# Some Effects of High Temperatures on the Perch PERCA FLUVIATILIS (Linnaeus) and their Importance in Influencing its Distribution

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SOME EFFECTS OF HIGH TEMPERATURES ON THE PERCH

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IN INFLUENCING ITS DISTRIBUTION.

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# I. INTRODUCTION

Brett (1956) reviewed many of the records which have been obtained in recent years of both upper and lower lethal temperatures for freshwater fish. Both he and Andrewartha & Birch (1954) among others have considered the ecological significance of such records. The latter writers have suggested that *"especially with certain species of fish which live in shallow lakes or rivers in temperate zones, high temperatures may sometimes cause a lot of deaths".* Brett, on the other hand considered that *"lethal (temperature) relations must play a relatively unimportant part in the limiting of many hardy species",* and suggested that *"Some other property or attribute roughly reflected by the upper lethal temperature must be operating as the restricting factor to distribution."* Hart (1952) also decided that upper lethal temperatures of fish species, as experimentally determined, may be correlated with the general thermal conditions characterising the waters in which they live, but that there is little indication that fish very often actually encounter lethal temperature conditions over their normal geographic ranges.

As regards Perca spp. - as represented, anyway, by P. fluviatilis (Linnaeus) and P. flavescens (Mitchill) - it is felt that this view of Brett and Hart may reflect an unsatisfactory posing of the whole question of the zoogeography of this genus. Upper lethal temperatures may influence distribution in a much more precise and direct manner than the above writers have thought. It is hoped to show that only a complete survey of the actual limits of zoogeographic distribution in relation to a suspected controlling factor (or factors) can lend necessary insight in appraising the role and importance of the factor.

Meanwhile it is true that in certain rather special instances heavy and obvious mortalities may result from exposure of fish to high temperatures in the field. The obvious kinds of places for such mortalities will certainly include the shallow temperate waters mentioned by Andrewartha & Birch (1954). Moreover, fish are especially prone to high temperature effects in waters where they can become trapped - as in the pools left along a drying watercourse in summer - from which they are unable to escape to less rigorous conditions. The operation of upper lethal temperatures might be characterised more generally - if somewhat tritely - by stating that a lethal effect is likely whenever fish are unable to avoid a prolonged exposure to a temperature higher

than that for which their level of acclimatization has prepared them. This may occur in shallow streams when the weather becomes suddenly hot for the time of the year as Huntsman (1946) demonstrated for Canadian Maritime stream fish populations. Sometimes non-indigenous fish species have been released in streams where they can survive in the summer only in the highland, or cooler stretches. Such fish may occasionally become trapped by rising temperatures on the fringe of their normal distribution, when large numbers may die; Lake (1957) documented an example of this for brown and rainbow trout in streams of New South Wales. In deeper thermally stratified lakes oxygen depletion in the hypolimnion during the summer may force fish upwards until they reach a temperature stratum beyond which they will not usually pass. Thus Fry (1937) demonstrated that the cisco Leucichthys artedi in Lake Nipissing, Ontario, left the bottom water and became concentrated just below the thermocline. And since this species normally avoids water of 20°C. or more the cisco population tended to remain just below the thermocline, held there by the impulse to escape from low oxygen and high carbon dioxide tensions in the bottom of the hypolimnion which was opposed by the impulse to avoid the steep temperature gradient above. As Fry

himself colourfully puts it: *"Their salvation lies in being able to stay within the frying pan until the fire has died away to embers."* But it is readily envisaged that should the careful poise of such fish be disturbed by, say, oxygen falling yet lower, then they could be forced to enter the thermocline where death could follow through heat, or a combination of heat and anoxia.

Fish ponds, and brackish water fish culture enclosures, form another kind of place in which fish often have little opportunity for evading high temperatures in summer, and are thus potentially dangerous environments, and D'Ancona (1954) has referred to the brackish water enclosures of the Adriatic in this connexion. Thus in fish culture it is sometimes desirable to determine the maximal temperatures likely to occur, together with the temperature tolerance of the species of fish with which it is proposed to stock the ponds or enclosures. The present writer measured summer temperature maxima in small Tasmanian farm dams, the potential of which as fish ponds was under trial, and found that temperatures were unlikely to exceed 25°C. (Weatherley, 1958). The fish used to stock these dams was the tench Tinca tinca (L.), and in experiments its upper lethal temperature was found to be about 35°C. when fully acclimatized to

Tasmanian summer conditions (Weatherley, 1959). It was thus concluded that it was very unlikely that tench would ever die from the simple effects of temperature in these dams, for even in winter (June to August in Tasmania) the upper lethal temperature of the species was about 27°C.

It is appropriate to mention at this point that many laboratory studies (Brett, 1956, collects together a large number of them) have shown lethal temperatures to be directly related to temperature of acclimatization. Where room for it still exists, upwards acclimatization to temperatures occurs more rapidly at high than at low temperatures, because acclimatization is a physiological process and, as fish are poikilotherms, is thus directly proportional to temperature.

In the search for a suitable conceptual approach to the question of heat impairment and death and associated problems, the scanty existing literature on heat-induced changes in fish tissues was considered. Several workers have reported for fish a depression or near cessation of metabolism of nerve tissue with the approach of upper lethal temperatures (Freeman, 1950; Fuhrman et al, 1944). Battle (1926) found that nerve tissue of Skates (Raja spp.) became inactivated at lower temperatures than did somatic and smooth muscle, though her methods seem rather arbitrary

and poorly designed to place much reliance on her results. Brett (1944) reported that respiratory movements of Perca flavescens ceased entirely as death approached. These studies give the impression that there is a temptation to invoke nervous failure as the weak link in a systemic physiological chain put into operation by dangerously high temperatures. In a sense this may be a valid viewpoint, yet tissues do not exist in the fish body as independent and isolated entities, but as parts of a functionally integrated system, whose activities reciprocally and intimately affect each other. Any strong (noxious) stimulus, continued long enough, will be likely to set in motion complex response mechanisms in the stimulated animal. One end result may be nervous impairment or failure, so that in a sense such failure might be said to cause death. But the problem of nerve disfunction would need to be viewed against a broad background of organ, tissue and functional change. Since for fish, knowledge of changes in the morphological integrity of any tissues, resulting from exposure to higher temperatures, appears to be almost non-existent, an examination biased in this direction seemed to be justified.

With this viewpoint in mind the author turned to the 'General-Adaptation-Syndrome' of Selye (1950), well-known

in medical endocrinology and latterly in clinical practice. This Syndrome has led to a broad theory concerning adaptive responses to noxious stimuli among mammals, which in spite of certain shortcomings which Selye himself has recognised (Selye, 1952), was considered to be of particular interest in the present investigation. Thus:-

(1) It points to the remarkable similarities of response called forth by numerous quite disparate noxious stimuli, applied to the animal for varying periods. These responses may usually be assessed by rather simple physiological and histological means. They include (a) marked changes in the adrenal cortex, lymphoid tissues and organs, and degenerative changes in the gut, kidney, spleen, liver and muscles, together with less reliable changes in the central nervous system, skeleton, and so on. All these changes are revealed histologically, or even by naked eye inspection in some cases. Changes may also be detected in the sodium and chloride balance, blood sugar and liver glycogen, proteins in blood and tissues, non-protein nitrogen in blood and urine, red and white cell blood counts and numerous other variables.

(2) Fish are sufficiently similar in structure and physiology to higher vertebrates to make it seem likely, a priori, that their systemic responses to prolonged noxious stimuli would somewhat resemble those of the latter.

Actually this had already been suggested in the work of others, notably of Dittus (1940), Hartman et al (1944), Rasquin (1951) and Rasquin & Rosenbloom (1954). Hoar (1957) has usefully reviewed the evidence.

(3) The General-Adaptation-Syndrome attempts to relate observed changes primarily to changes in the rate of secretion of the pituitary, secondarily, to a large extent, to changes induced in the activity of the adrenal cortex through the increased pituitary activity. Such an approach lends itself to histological appraisal as exemplified in the work of Selye and his colleagues, and to simple experimentation along endocrinological lines.

Early experiments seemed to affirm that this would be a profitable approach, so the course of the investigation has been as indicated above, with emphasis on the histological side.

# II. MATERIALS AND METHODS

## (a) The Fish

### (1) Sources

Perch Perca fluviatilis (L.) for the early experiments on lethal temperatures came from Loch Lomond, the principal body of freshwater in Scotland, where they were seine-netted or trapped. These fish showed a considerable tendency to be mildly infected with fungus (Saprolegnia sp.) when captured, and the infections often became serious under aquarium conditions. During winter it is hardly possible to capture perch in Loch Lomond, for they migrate into deeper water as in Lake Windermere (Allen, 1935) and other large lakes. These disadvantages, together with their usually large size, made Loch Lomond perch rather unsatisfactory for experiments, so that after the initial experiments they were no longer sought there.

For experiments with a histological evaluation, and for injection experiments, fish were obtained from Dumbrock Reservoir near Glasgow, by seine netting and trapping. These were particularly healthy looking fish and did not develop fungus infections if they were undamaged at time of capture. They generally showed no ill effects from aquarium life even after a prolonged period in stock tanks.

(11) Prophylaxis and feeding

As soon as possible after capture fish were always brought to the Zoology Department's aquarium and placed in a stock tank until required in experiments. Though compounds such as formaldehyde, sodium choloride, propylene phenoxytol and methylene blue were tried as therapeutic agents in treating perch infected with fungus, none was really very useful once fish were seriously infected. After the difficulties with the Loch Lomond fish all perch were exposed as a routine to a treatment with methylene blue dissolved in the water in a concentration sufficient to make it difficult to see the fish in diffused daylight. It is not certain that this treatment, repeated about once every ten days for some batches, was particularly effective in controlling the development of fungus, though it was practically never seen again after the institution of this practice. It seems more likely, indeed, that the failure of fungus to develop was due to its virtual absence from the fish when taken in Dumbrock Reservoir.

Fish were fed freely, usually every day, on a food made up as follows:-

'Lassie' dog food ..... 200 g.

Gelatine (10% aq.) ..... 200 g.

Dried shredded shrimp ..... 10 g.

'Bemax' ..... 5-10 g.

Appendix Table 1 gives the composition of 'Lassie' and 'Bemax'. It is sufficient to observe here that the fish were, by this diet, provided with a considerable range of proteins, essential amino acids, fats, carbohydrates, minerals and vitamins, and that they appeared to live healthily on it, with no obvious loss of normal colour or vigour.

(b) Tanks

All experiments were carried out in glass aquarium tanks. Two of these, measuring 3 ft x 2 ft x 2 ft, and 3 ft x 1½ ft x 1 ft, respectively were larger than the others. These were used as stock tanks and it was into them that fish were placed on arriving in the aquarium, and where they were always left for at least a week before use in experiments. This period was intended to allow the fish to settle down to life under aquarium conditions and also to recover from any stresses accompanying their capture and transport thither. Perch which were not in acclimatization or lethal temperature tanks remained in the stock tanks until required. The population density in the stock tanks varied greatly during the course of the experiments, but the greatest density was about one hundred of the smaller perch (Table 2) or about 80 of the larger perch which were used in the second series of

## Table 1.

*Analysis of water as supplied to Zoology Department, Glasgow University. Values in p.p.m., except for pH and colour.*

| | |
|---|---|
| Free and saline nitrogen | 0.002 |
| Albuminoid nitrogen | 0.025 |
| Oxygen absorbed from permanganate in 15 min. at 27° C | 0.53 |
| Oxygen absorbed from permanganate in 4 hr. at 27° C | 0.93 |
| Chlorides (as Cl) | 8 |
| Nitrates (as N) | 0.10 |
| Nitrites (as N) | Nil. |
| Total solids | 32 |
| Mineral solids | 18 |
| Organic solids | 14 |
| Total hardness (as CaCO3) | 9.0 |
| pH | 6.0 |
| Colour (Hazen units) | 10 |
| Free Cl | Nil. |
| Chloramine | Nil. |
| Hardness (E.D.T.A. method) | |
| Calcium hardness (CaCO3) | 6.0 |
| Magnesium hardness (CaCO3) | 3.0 |
| Total hardness (CaCO3) | 9.0 |
| | |
| Calcium (Ca) | 2.13 |
| Magnesium (Mg) | 0.63 |
| Iron (Fe) | 0.01 |
| Sodium (Na) | 3.89 |
| Manganese (Mn) | 0.006 |
| Aluminium (Al) | 0.04 |
| Zinc (Zn) | 0.012 |
| Silica (SiO2) | 0.75 |
| Sulphate (SO4) | 4.16 |
| Phosphate )PO4) | 0.0001 |
| Chloride (Cl) | 8 |
| Fluoride (F) | 0.09 |
| Iodide (I) | 0.008 |

*By courtesy of Corporation Chemist's and City Analyst's Department, Glasgow.*

*The values in the above table are for June 1959. They may be considered representative, as monthly variations are only slight.*

experiments (Section V).

Smaller tanks measuring 2 ft x 1 ft x 1 ft were used for acclimatization experiments. In these experiments the number of fish per tank did not exceed two dozen fish, and was usually less than twenty. During the second series of experiments (Section V), in which the larger perch were used, the greatest number of fish per tank was 20. The lethal temperature tests were also carried out in these smaller tanks.

All save the larger stock tank were supplied with running water, the flow of which was adjustable. The water, coming from the Glasgow City Mains, has the chemical composition shown in Table 1. The water in all tanks was continuously aerated by means of compressed air led through rubber tubes terminating in aquarium airstones.

The tanks usually had some washed quartz gravel, collected from a Loch Lomond beach, covering the bottom. This acted partly as a trap for food wastes, which were easily removed from it by suction when the tanks were being cleaned, and also provided a more 'natural' substratum for the perch than a bottom of plain glass.

Those tanks used in acclimatization experiments were heated with aquarium heaters of 70-120 watts, fastened

together in batteries of three or four on perspex sheets; temperature was contolled to about 1°C. around the level selected by means of 'Prockter' external thermostats. For heating the water in the tanks used in lethal temperature experiments a 'Tempunit' 1000 watt thermostat-heater unit was employed. The heating coil of this unit, which surrounds a propellor-stirrer, was guarded during experiments by a cylinder of perforated perspex to protect the fish from contact with the stirrer blades. The 'Tempunit' was also used in a few acclimatization experiments when it gave temperature control of the order of 0.1°C. around the selected level.

The tanks were initially equipped with cardboard screens and later with opaque green cloth curtains on three sides to protect the perch from the visual stimuli of outside movements in the aquarium. It had been noted quite early that such movements often caused marked excitement, but cautious examination of the fish behind the screens always revealed them to be very quiescent at lower temperatures. Only at acclimatization temperatures of about 25°C. or more did they swim fairly freely around their tanks. The uncovered side of the tanks faced the aquarium windows and was never approached.

No attempt was made to illuminate the tanks artificially

or to interfere with the normal passage of day to night. Light for the aquarium came from windows and skylights, and artifical light was used only as occasion demanded during observations accompanying experiments, or when it was necessary to work in the aquarium at night.

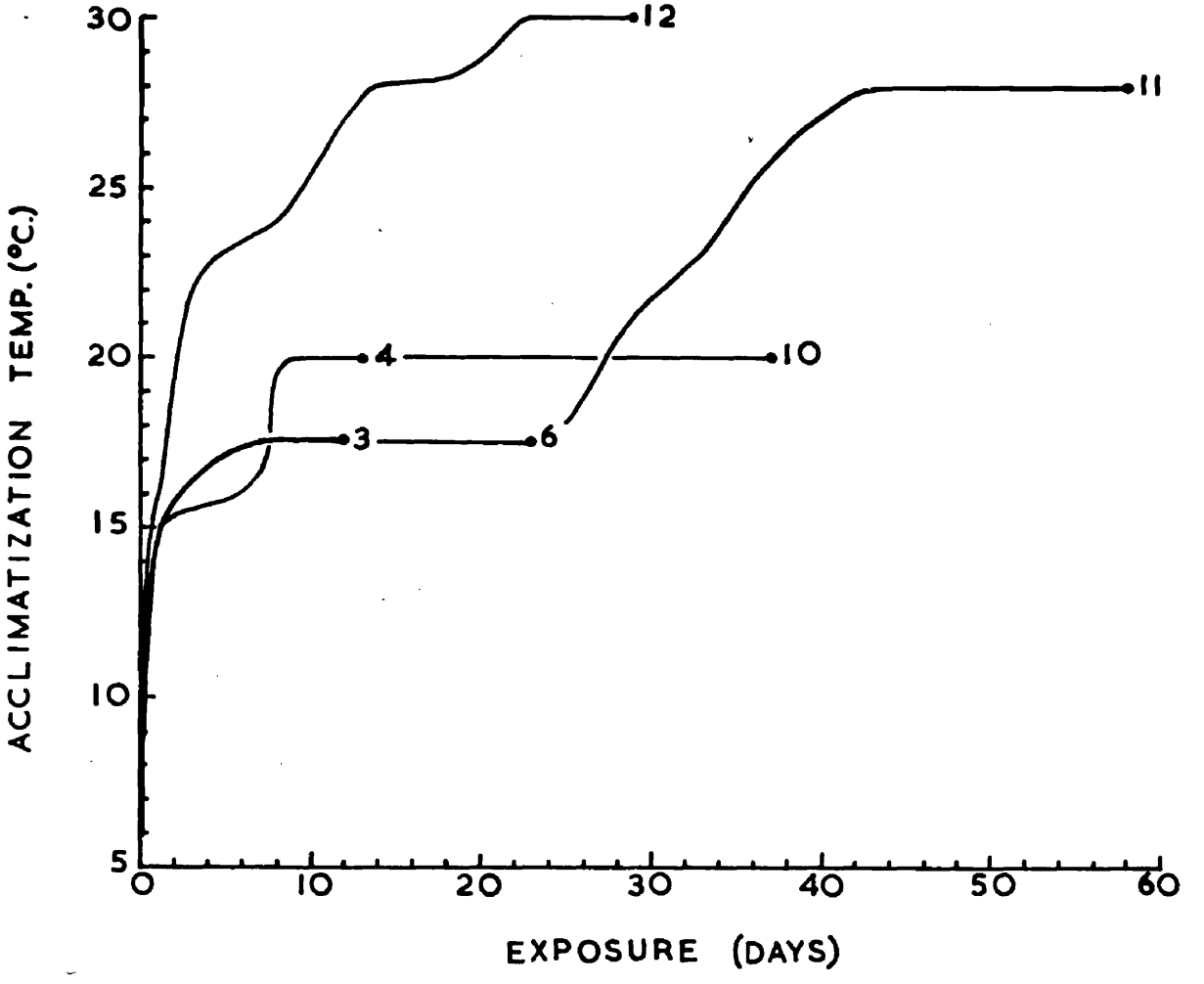
(c) Acclimatization Procedures and Lethal Temperature Experiments

In the stock tanks in which the fish were first placed after capture water temperature was allowed to vary naturally just as it came from the mains. However, over any period of a few consecutive weeks in winter, temperature normally varied by no more than $\pm 1^{\circ}C$. and was often more constant than this for weeks at a time. In spring and summer conditions were more variable.

In acclimatizing a group of fish to a temperature above that of mains water they were removed to one of the smaller tanks where the temperature was lifted, at rates which varied between experiments, until the required temperature was achieved (Figure 1). Duration of exposure to this temperature varied with requirements as will be seen.

Determinations of upper lethal temperature were performed in the smaller tanks by raising the temperature to the lethal level at the rate of $4^{\circ}C$. per hour. The mean temperature at death of the batch was taken as the

Figure 1. Curves of acclimatization (water temperature versus period of exposure) for perch in aquarium tanks in Experiments 3,4,6,10, 11 & 12. The black dots adjacent to the numerals indicate the points at which the experiments were terminated (by a lethal test). See text for further details.


30
25
20
15
10
5
ACCLIMATIZATION TEMP. (°C.)
0
10
20
30
40
50
60
EXPOSURE (DAYS)
12
11
4
10
3
6

lethal temperature. A substantially similar method was adopted by the present writer in determining upper lethal temperatures of the tench Tinca tinca (L.) (Weatherley, 1959).

(d) Histological Procedures

Structures examined histologically included head kidney, interrenal tissue, kidney, liver, spleen, muscle, thyroid and central nervous tissue. As a routine sections were cut at 4$\mu$ or 6$\mu$; mostly at the former.

Fixation was usually effected by placing the whole fish in Bouin's fluid. By slitting open the fish ventrally along the length of the body cavity satisfactory fixation of all required organs was obtained. Following fixation all tissues were stored until needed in 70% alcohol.

With the small perch used in this work it was not possible to dissect out head kidneys and kidneys without damage. It was found best to cut a block of tissue containing these organs, backed and strengthened by the vertebral column and some muscle. A section cut of such a block of tissue would give an overall view of several important tissues at once, a procedure economical of time and effort. But of course, decalcification of these and the head blocks (for brain and thyroid) was

necessary before wax infiltration, and this was carried out by immersing the blocks in 5% nitric acid in 70% alcohol, or in a mixture of 8N formic acid in N sodium formate. Neither decalcifying agent seemed superior to the other, and about 2 to 4 days were usually required for complete decalcification.

When removed from the fish livers and spleens were embedded in polyester wax (Steedman, 1957), but the decalcified blocks of tissue, after repeated washings in 70% alcohol were infiltrated with ester wax (Steedman, 1947).

Haemalum and eosin were found suitable as general purpose stains, especially where it was required to stain a number of different tissues in each section (and often serial sections of a given region) with sufficient differentiation and clarity both for ready separation and identification, and to make out something of fine structure. Mallory's trichrome was tried a number of times but found less generally satisfactory.

(e) Experimental Procedures

The main experiments were designed primarily to supply information on the manner of heat impairment and the events leading to death. Of the dozen performed to this end ten are set out in summary in Table 3. It can be seen that they fall into three groups.

The first natural group comprises fish which were kept for a considerable time (several weeks) at the low acclimatization temperature of 7.5 or 7.6°C. Moreover, it should be noted that they came from Dumbrock Reservoir when the temperature was only a degree or so above this level. As indicated in Table 3 a number of fish in this group were raised to their lethal temperature at the rate of 4°C. per hour.

The second group comprises perch which were acclimatized to 17.5°C. and 20°C. which are thought of as moderately high temperatures, being something of the order which might be encountered in cooler, temperate, surface waters during the summer. As table 3 and Figure 1 show, the perch were exposed (acclimatized) to these temperatures for various periods before lethal testing.

The fourth natural grouping represents fish acclimatized to what were definitely 'high' temperatures. Of the batch acclimatized to 28°C. it can be said that all seemed in good health, though a few died on the way up to this temperature. Much the same applies to the 30°C. batch.

Figure 1 shows the thermal backgrounds in the aquarium of perch in Experiments 3, 4, 6, 10, 11 and 12.

Whenever a lethal temperature test was performed on fish from the acclimatization tanks a few were fixed before

the test began for future histological examination. Fish were also taken from the lethal temperature tanks just before disablement and immediately after death, and as controls to all experiments fish were removed from the low-temperature stock tank and fixed each time an experiment was performed. In the results to follow the four categories may be referred to as follows:-

Low temperature controls: those coming from the low-temperature stock tank;

Experimental controls: those taken from the acclimatization tanks immediately before the beginning of a lethal temperature experiment;

* Fish before disablement: those fish removed just before disablement during a lethal test;

\+ Fish after death: those removed immediately after death.

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* *These fish were removed from the lethal test tank immediately after the first fish in the sample had shown signs of disablement, though they were not themselves disabled.*

\+ *It must be emphasised that throughout this study, with the exception of perch in Experiment C, Section V (a) (v), when it is stated that fish were removed after death during a lethal test it is meant that they were taken from the tank as soon as movement had ceased and placed directly into Bouin's fluid. Hence, any differences between the histology of such fish and those fixed before disablement cannot be due to post mortem changes, but must have occurred in the small period of time (and of rising temperature) which elapsed between the brink of disablement and death.*

The weights and total lengths (Le Cren, 1947) of all the fish involved in these experiments were recorded. The means and ranges of these data are given in Table 2. These small fish were all sexually immature and it was not possible to sex them by ordinary internal examination.

Further information on methods employed in experiments arising out of the acclimatization series (Section IV) is given in Section V. Larger fish were employed in this second group of experiments, because it did not prove possible to capture any more of the very small fish used in the first series; but the source of the fish was again Dumbrock Reservoir.

## Table 2.

**Lengths and weights for perch in experiments yielding histological material and upper lethal temperature data.**

| Source of fish | Experiment no. | No. fish | Length (cm.) | | Weight (g.) | |
|---|---|---|---|---|---|---|
| | | | Mean | Range | Mean | Range |
| Dumbrock Reservoir | 1 | 8 | 6.7 | 6.3 - 7.0 | 2.5 | 1.9 - 3.1 |
| | 3 | 16 | 6.2 | 6.0 - 7.0 | 1.8 | 1.3 - 2.8 |
| | 4 | 23 | 6.1 | 4.7 - 7.3 | 1.7 | 0.6 - 3.2 |
| | 5 | 12 | 6.2 | 5.3 - 7.0 | 1.7 | 1.0 - 2.6 |
| | 6 | 18 | 6.0 | 5.3 - 6.4 | 1.5 | 0.9 - 2.1 |
| | 7 | 11 | 6.2 | 5.7 - 6.6 | 1.8 | 1.2 - 2.4 |
| | 10 | 13 | 5.9 | 5.5 - 6.4 | 1.4 | 0.9 - 2.0 |
| | 11 | 12 | 6.2 | 5.8 - 7.1 | 1.6 | 1.2 - 2.2 |
| | 12 | 9 | 6.0 | 5.6 - 6.4 | 1.4 | 0.9 - 2.0 |
| | Low temp. controls | 21 | 6.3 | 5.4 - 7.6 | 1.9 | 1.0 - 3.6 |
| Tasmania | Expts 1-5 | 19 | 21.1 | 16.4 - 26.0 | 127.8 | 65.2 -242.3 |

## III. THE ROLE OF HIGHER TEMPERATURES IN LIMITING DISTRIBUTION OF PERCH

### (a) Results of Upper Lethal Temperature Experiments

#### (i) Upper lethal temperature and acclimatization temperature

Of the lethal temperature experiments performed as described in Section II six are represented graphically in Figure 2. Here mean upper lethal temperatures have been plotted against temperatures of acclimatization for Experiments 1,5,6,10, 11 and 12. The plotted values reveal the direct relationship between these variables frequently described for many species of freshwater teleosts (e.g. by Brett, 1944, 1952, 1956; Fry, Hart & Walker, 1946; Hart, 1952; Cocking, 1959a). Table 3 shows the data from which Figure 2 was constructed, and indicates the range of lethal temperatures (as well as the mean lethal temperature) for each experiment. Only those results were selected for plotting in Figure 2 which came from tests carried out on fish deemed thoroughly acclimatized to a particular temperature (see Figure 1 and Table 3).

Figure 2 also contains values obtained on Tasmanian perch. In Tasmania, Perca fluviatilis is an introduced, though flourishing, species, and these fish came from a perch population established by the present writer

Figure 2. Upper lethal temperature versus temperature of acclimatization for perch. See Table 3 and text for further information. Values for Scottish fish, black circles; for Tasmanian fish, open circles.

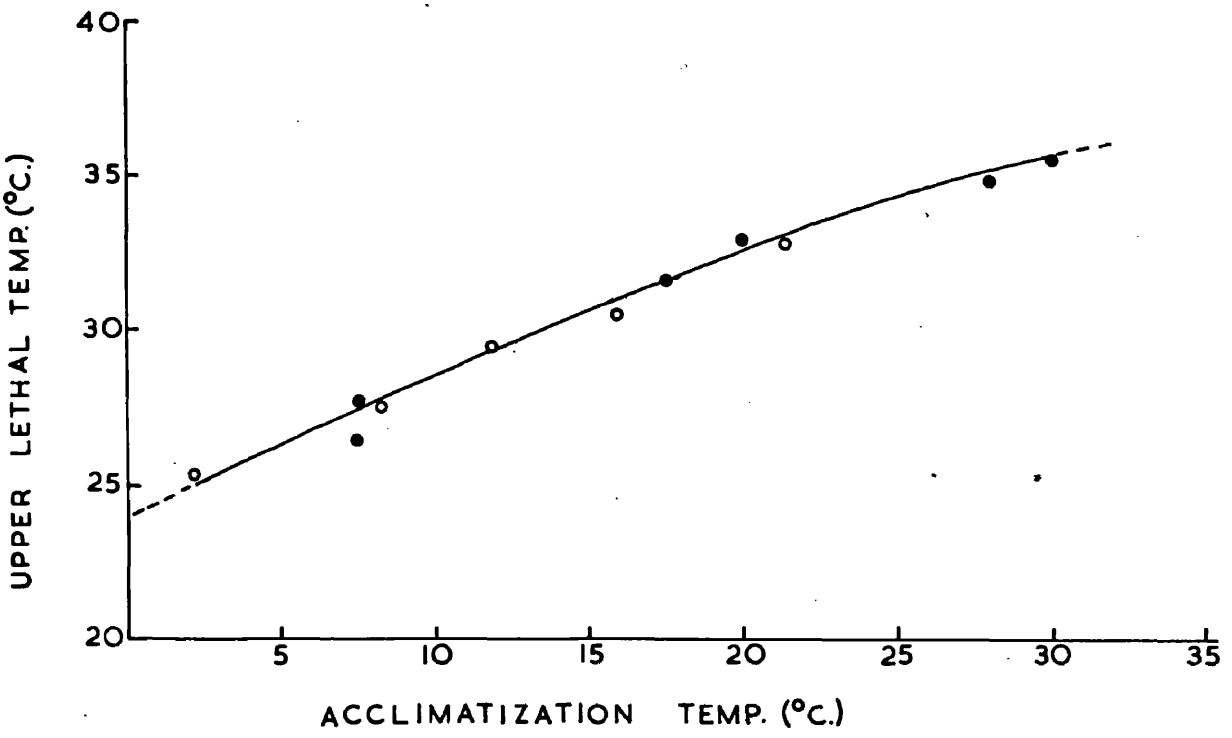

40
35
30
25
20
UPPER LETHAL TEMP. (°C.)
5
10
15
20
25
30
35
ACCLIMATIZATION TEMP. (°C.)

*Table 3.*

*Experiments yielding histological material and upper lethal temperature data for Perca fluviatilis.*

| Source of fish | Experiment no. | Acclimatization temp. (°C) | Duration of acclimatization | No. fish acclimatized | Mean upper lethal temp. (°C) | Range (°C) | No. fish on which lethal temperature is based |
|---|---|---|---|---|---|---|---|
| Dumbrock Reservoir | 1 | 7.6 | several weeks | 8 | 27.7 | 26.2 - 28.2 | 8 |
| | 5 | 7.5 | " " | 12 | 26.4 | 26.0 - 27.2 | 8 |
| | 7 | 17.5 | 2 days | 11 | 30.0 | 29.4 - 31.2 | 7 |
| | 3 | 17.5 | 7 " | 16 | 29.2 | 27.7 - 30.0 | 8 |
| | 6 | 17.5 | 13 " | 18 | 31.6 | 31.0 - 32.0 | 10 |
| | 8 | 20 | 1 " | 12 | 29.7 | 29.0 - 30.0 | 12 |
| | 4 | 20 | 6 " | 23 | 31.7 | 30.6 - 32.0 | 11 |
| | 10 | 20 | 30 " | 13 | 32.9 | 32.6 - 33.3 | 5 |
| | 11 | 28 | 16 " | 12 | 34.8 | 32.2 - 36.0 | 8 |
| | 12 | 30 | 7 " | 9 | 35.5 | 34.7 - 36.3 | 5 |
| Tasmania | 1 | 2.2 | 2 weeks | - | 25.3 | 25.2 - 25.5 | 5 |
| | 2 | 8.3 | " " | - | 27.5 | 26.9 - 28.0 | 3 |
| | 3 | 11.9 | " " | - | 29.4 | 29.1 - 29.7 | 3 |
| | 4 | 15.9 | " " | - | 30.5 | 30.1 - 30.8 | 3 |
| | 5 | 21.4 | " " | - | 32.7 | 32.6 - 33.0 | 5 |

in a large hatchery pond in southern Tasmania, from fish that he had captured in the field. (see also Tables 2 and 3). Lethal temperatures were determined on these perch at various times of the year, using a method much the same as that described in Section II and as given for tench (Weatherley, 1959). Temperatures of acclimatization were calculated as the mean temperatures in the pond during the two weeks immediately preceding each lethal test. These mean values were calculated from routinely recorded daily maxima and minima in the pond. The normal daily range in winter was only about $1^{o}$C. and in summer no more than $2-3^{o}$C. The trend of these upper lethal temperatures against acclimatization temperatures is remarkably similar to those for Dumbrock (Scottish) fish, and provides an additional point for acclimatization temperatures below $5^{o}$C. Though these Tasmanian fish formed only a small sample, representing just a few specimens available from a pond population with other primary uses, yet the small range of lethal temperatures for each experiment suggests that the values in Figure 2 are reasonably valid. Therefore, in addition, the values give some assurance that the acclimatization methods practiced in the aquarium yielded valid data.

Of course, Figure 2 implies that wild fish will die at lower temperatures in winter than in summer, as Brett (1944) clearly showed for several species of fresh-water fish collected from the field immediately before testing. A similar phenomenon has also been found for the tench Tinca tinca (L.) (Weatherley, 1959, and Figure 3). That it does in fact apply to the perch is illustrated in Figure 4. Here, values obtained for lethal temperatures of Tasmanian perch have been arranged according to time of year at which they were determined, with the mean temperatures for the months shown by means of histograms. These values, then, signify the change in upper lethal temperature in one population of perch in a pond over a period of many months, during which water temperature changed seasonally in a natural manner. The water supplying this pond came from the adjacent Plenty River, and the pond's temperature closely approximated that in the river.

The Tasmanian perch were much larger than those used in the Scottish experiments (Table 2), so that size is unlikely to be significant in influencing upper lethal temperature. It is interesting to note in this connexion that Hathaway (1927) and Hart (1952) detected no significant difference in upper lethal temperature between size groups of the North American yellow perch Perca flavescens.

Figure 3. Temperatures of disablement and death for tench at various times of the year. This is Figure 1 from Weatherley (1959).

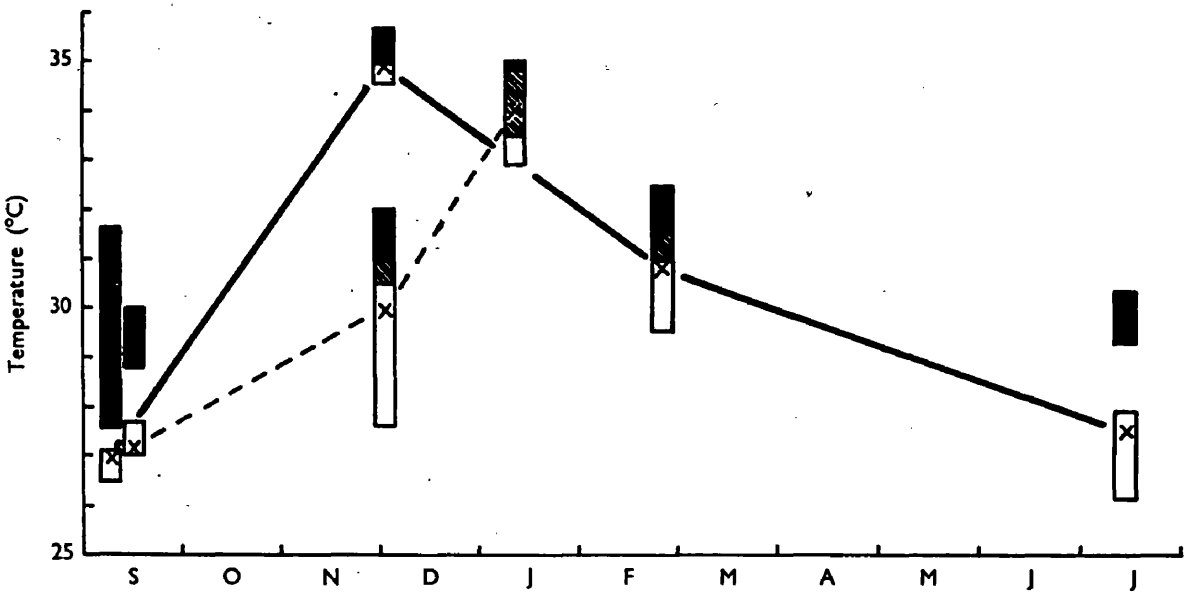
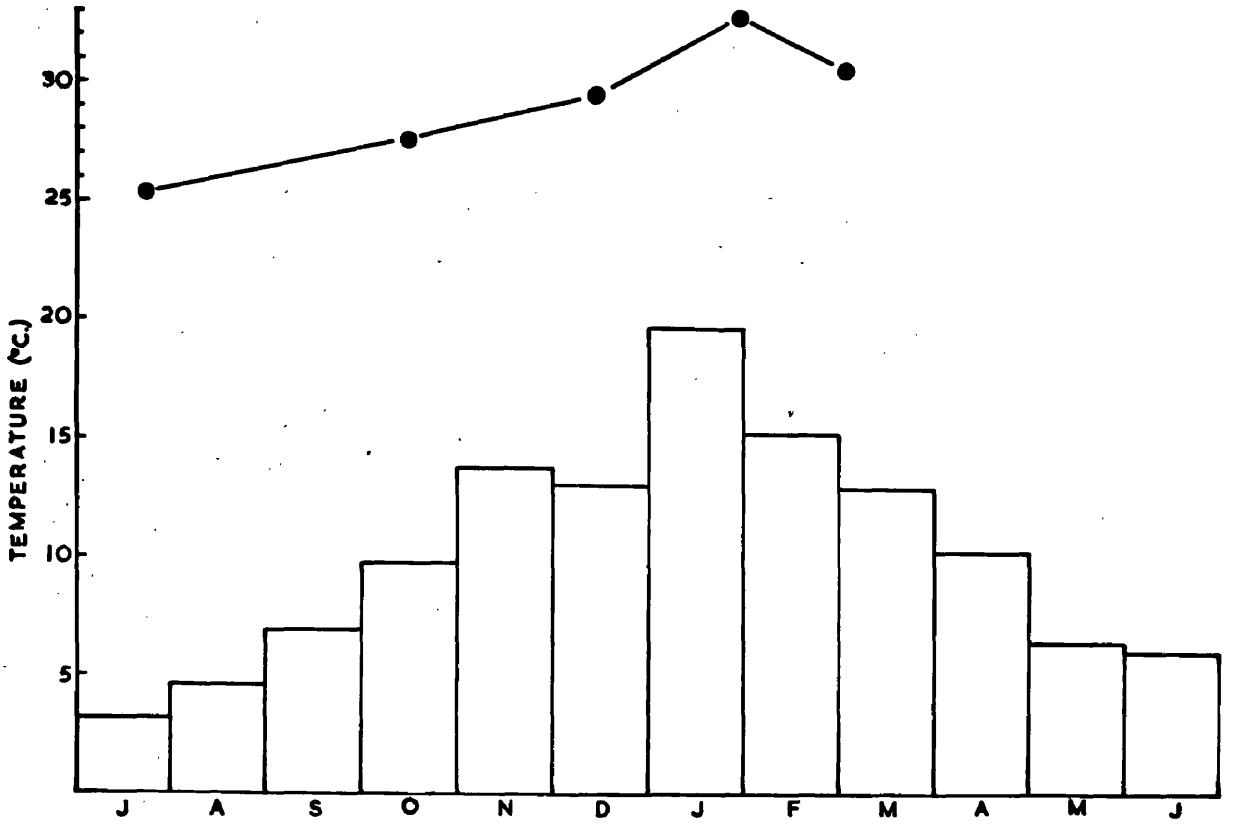



Fig. 1. Temperatures of disablement and death for tench at various times of the year. White columns represent the range of temperature over which fish became disabled; black columns indicate the range over which the disabled fish died; hatched columns indicate where the two overlapped. × signifies the mean temperature of disablement for a given batch of fish; —, fish from L. Tiberias; — — —, fish from Coal River.

Figure 4. Curve of seasonal variation in upper lethal temperature for Perca fluviatilis in Tasmanian hatchery pond supplied with running river water. Histograms represent mean daily temperature for each month of year.


TEMPERATURE (°C.)
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Fry (1958) has suggested that an absence of relation between size and heat death may signify *"that heat death is likely to be due to the breakdown of certain critical cells,"* which is a way of re-stating the belief of Brett (1956) that nervous tissue may be fundamentally involved. But such a suggestion seems to be a non sequiter, since there are really only two possible views about heat death: either it follows the *"breakdown of certain critical cells"* or the system as a whole becomes defective, in which case, as an accompaniment or consequence, cell breakdown may, certainly, occur. But there appears to be no more reason why the vulnerability of certain cells should be unrelated to size than that systemic degeneration should; nor, of course, is there any obvious reason why they should be related.

Some early lethal temperature tests were performed on Loch Lomond perch, but for several reasons they were considered unsatisfactory and are not included here.

(ii) Behaviour of perch during lethal temperature experiments.

When the temperature of the water is being raised, in an experiment, from a given acclimatization temperature perch vary somewhat in individual behaviour, but in general their behaviour is typified in the following account:-

If the starting temperature be low (5-10°C.) the fish will not visibly react much to the normal elevation of 4°C. per hour until the water temperature has been raised 10-15°C. above the initial level. Then they begin to move around the lower part of the tank more or less freely, sometimes exhibiting considerable mobility, at others becoming quiescent at the bottom, usually for a short period, even though temperature continues to rise, and though they may have been moving freely just previously. This kind of pattern tends to be repeated right up to the time and temperature when actual lethal effects supervene, though their movements become increasingly rapid and seem to be more desperately 'exploratory' as the lethal level is approached. As these latter stages draw near many of the perch probe into every corner and crevice of the tank and several may often be seen jostling each other in the corners. About this time, at least some of the fish begin to make momentary visits to the surface from time to time.

The first 'sign of disablement' is very similar to that for tench (Weatherley, 1959) and involves loss of balance and co-ordination. Such a condition may prevail for some time, beginning gradually and increasing steadily until total unco-ordination and immobility result;

or it may pass directly and almost immediately into this final phase. But the change from slight functional impairment to death (total cessation of movement) is, anyway, usually much more rapid for perch than for tench (Weatherley, 1959), so that the difference between that temperature causing disablement and that at the point of death, which were distinct enough to justify separation on the graph in the case of tench (Figure 3), are very slight and not worth separate recording for perch.

During the period that perch are impaired but not yet badly disabled they may continue to cruise, often on their sides or upside down, occasionally darting spasmodically but violently around the tank, behaviour which again resembles that of tench. In addition, Ameiurus, Semotilus, Marganiscus, Notemigonus and Perca flavescens (Brett, 1944) and Rutilis (Cocking, 1959a) appear to behave in a similar way when approaching thermal death, so this kind of behaviour is probably general for teleosts under these conditions. At the point of heat death perch lose the dark, vertical bars, which are so characteristic a feature of their colouration during life. Cocking, (1959a) described the development of a black pattern in the roach during the final stage of distress before heat death.

Fish which have been acclimatized to higher temperatures (say 20-30°C., especially above 25°C.) behave somewhat differently when the subjects of a lethal test. Their activity is already higher, for perch held at temperatures much above 20°C. move about more freely and actively at all times than those held at lower temperatures. Indeed, at 6-7°C. they either merely maintain their position in the water or rest upon the bottom, with minimal activity unless moving after food or when disturbed. However, when fish acclimatized to 25°C. or more are exposed to the rising temperature of a lethal test their freedom in swimming changes but little with increasing temperature, since they will already have been swimming fairly actively at the beginning of the test.

While the curve in Figure 2 shows that perch acclimatized to 30°C. may survive temperatures several degrees above this for a short time at least, it is more important to observe here that during this study two groups of fish were acclimatized to 29 and 30°C. respectively and in each case, apart from their high activity, they seemed completely healthy by superficial examination. Even colour was much as normal, though somewhat lighter. Yet elevation to temperatures even of 1-2°C. above 30°C. for more than a few

hours killed off a part of the fish in one case, and in the other case the whole batch.

Thus the strong impression emerges that though perch could tolerate temperatures up to 30°C. in the laboratory, given an appropriate acclimatization background, tolerance of temperatures in excess of this level was possible only for very limited periods.

The data of Hart (1952), though obtained from rather differently designed experiments, leads to a closely similar impression of the thermal tolerance of Perca flavescens, the North American species which is such a near relative of Perca fluviatilis.

(b) Zoogeography of Perch with Special Reference to the Effects of Water Temperature

(i) The background to the problem

Brett (1956) has claimed for freshwater fish that while the *"highest seasonal lethal temperatures recorded for ..... six species ranged from 32°C. to 35°C., yet the full scope for temperature tolerance had not been tapped, as laboratory experiments revealed. The ability to exceed considerably the highest environmental temperatures to be expected in the common range of the species signifies that lethal temperature relations must play a relatively unimportant part in the limiting of many hardy species. Yet, in their general order of increasing heat tolerance,*

*they show a correlation with an ecological succession from cold streams and deep lakes to warmer streams and littoral zones, and finally to marsh conditions ..... Some other property or attribute of the organism, roughly reflected by the upper lethal temperature must be operating as the restricting factor to distribution*".

Brett goes on to claim that there are but few recorded instances of heat death in the literature (see Introduction). However, such negative evidence seems a weak criterion, since it is notoriously difficult to demonstrate, except by inference, the extent to which a given factor of supposed importance is producing lethal or limiting effects on a species, except, for instance, in cases of direct and obvious predation or disease. Brett also refers to the failure of tested species to encounter temperatures *"in (their) common range"* which are lethal to them under laboratory conditions. But this may be because it is not in *"the common range"* of a species that one would look for or expect to find a lethal temperature, but rather at the latitudinal extremes of that range. And it is hoped to demonstrate here that it is untenable to suggest, at least for Perca spp., that high temperature has little connexion with observed limits of distribution.

A principal weakness of the approach of Brett (1956) and Hart (1952) to this question of distribution and high temperatures is that they refer to *'typical habitats'* of various fish species, including Perca flavescens. Brett lists the habitats of this species as *"deep and shallow lakes."* Such information is both vague and inadequate, and of course embraces a huge range of environmental types, while telling nothing concrete about the zoogeography of the species, while a fundamental criticism of this habitat designation is that perch are, after all, commonly found in rivers of a certain type as well as in lakes.

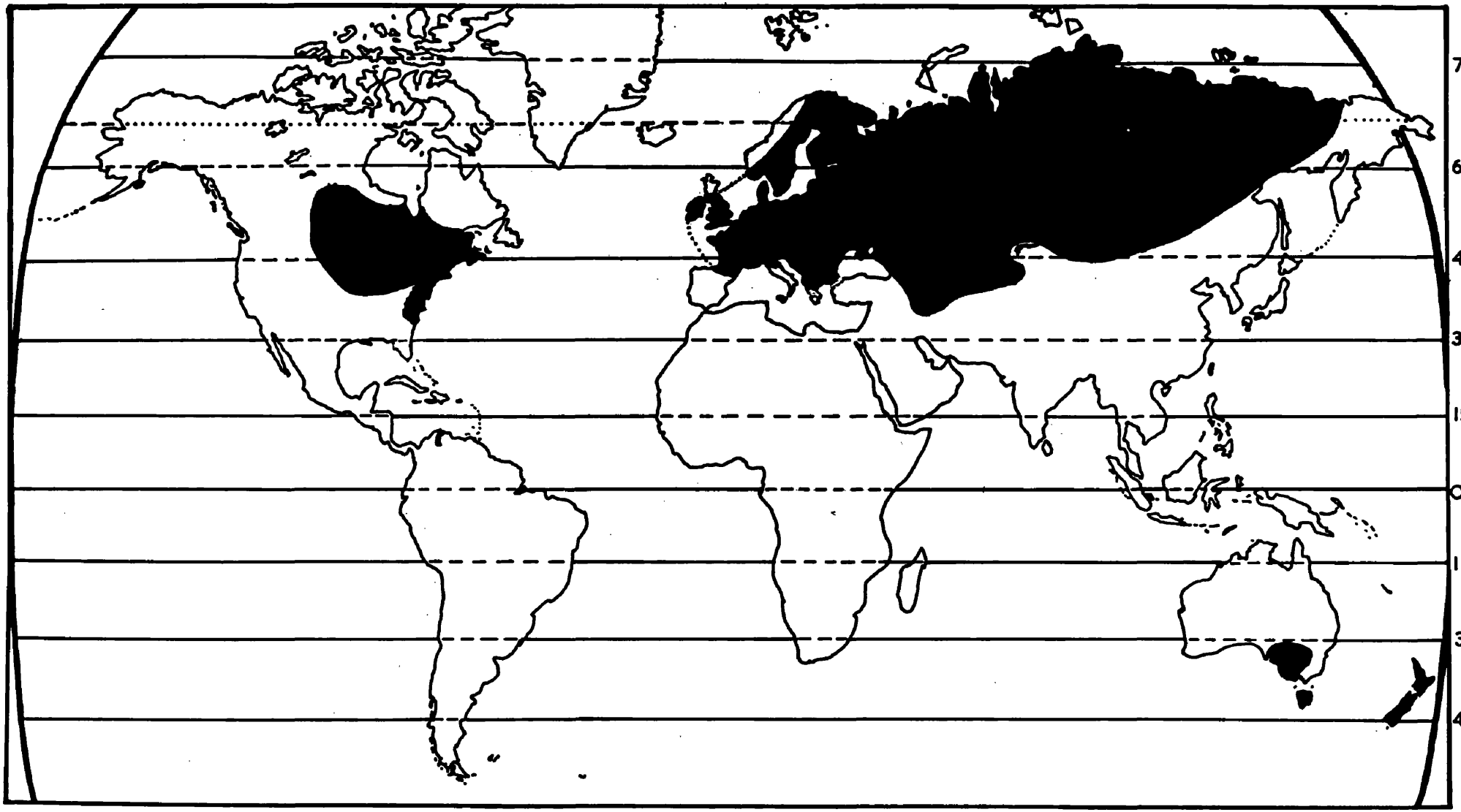
In considering the zoogeography of a species, with a view to estimating the importance of a factor in imposing distributional limits, it is first necessary to determine the actual limits of the distribution as precisely as possible. In addition, the fuller the appreciation of the general biology of the species under consideration, and the more complete the knowledge of local or regional barriers to distribution, the more precisely will it be possible to test the effectiveness of the factor deemed important.

In the present instance the task is simplified in several ways. Thus Perca fluviatilis has at present a

wide distribution, being found in Europe and Asia and also, through the agency of man, in Australasia and South Africa as well (Figure 5). Except for South Africa it was introduced into the southern hemisphere in the 19th Century. So it is apparent that an hypothesis concerning the perch's distribution may be tested over a considerable fraction of the earth's surface. Again, the biology of P. fluviatilis has been so extensively studied by numerous biologists that its life history, habits and behaviour (Maxwell, 1904; Schindler, 1957), growth (Alm, 1946, 1954; Deelder 1951; Le Cren, 1947, 1958), food (Allen, 1935), breeding cycles (Le Cren, 1951, 1958) seasonal movements (Allen, 1935), etc., are among the better known of these for freshwater fish. The yellow perch, P. flavescens, in North America is an equally well-known species, whose general habits coincide almost completely with those of P. fluviatilis. And not only are these two species of the same genus, but their connexion may be even closer. Thus Günther (1880) wrote that P. fluviatilis was *"generally distributed over Europe and Northern Asia; and equally common in North America, there being no sufficient ground for separating specifically the specimens of the Western Hemisphere."* Of more recent authorities Berg (1948-9) suggested that P. fluviatilis of north-eastern Siberia were transitional to what he

Figure 5. World distribution of perch. Perca flavescens is restricted to North America. Perca fluviatilis is found in Britain, Eurasia, Australasia and South Africa, though in the latter the extent of its distribution is apparently not accurately known. Main areas of occurrence are shown in black.

This figure was constructed from the data of numerous authors referred to in the text.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considers as the American subspecies. On the other hand, Jordan & Gilbert (1882) indicated that there were really more differences between the two stocks than had often been acknowledged, and listed some of these. While most modern authorities seem to assume a valid separation of the species their remarkable similarity of appearance and habit is acknowledged.

It is apparently impossible to obtain any help from paleontological evidence in deciding this and related problems, for though a Northern Hemisphere origin for perch may be assumed and the records seem to suggest their presence at least as far back as the Pleistocene (e.g. Leriche, 1951), it is not, beyond this, possible to localise their origin with certainty though Macfarlane (1923) argues persuasively for a North American origin, basing his beliefs on the relative abundance there of Percid genera and species.

Perch are not usually found in the oligotrophic highland lakes where, for instance, Salmonid spp. so often find a suitable habitat. Deep and shallow lakes alike, of a more eutrophic character, often contain them. Pearsall (1949) has pointed out that in the English lakes a perch-pike association is often found in the more silted (evolved) waters. In rivers they rarely occur

in short, steep, rapid streams where, as in oligotrophic lakes, Salmonids often flourish. Rather are they found in slow, mature streams, especially in the meanders, holes, ox-bows and backwaters of these. Indeed, it is conspicuously true, as the writer found in Tasmania, that even in a stream in which perch are abundant they may have a remarkably discontinuous and localised distribution. Thus they could be reliably trapped in many of the quiet and stagnant sections of such a stream, but where the rate of flow was more rapid, even if this was only about 30-40 yards from the successful trapping site, hardly any perch could be taken.

Perch deposit their ova in weed beds or on other suitable substrata such as submerged sticks or rocks, to which the adherent strings of eggs are attached, but only where such substrata are found in the shallows of lakes and in quieter stretches of rivers. The swimming powers of perch are evidently inadequate for them to live in the open waters of a swift-flowing river, and this, coupled with their tendency to school and to spend much of their time quiescent close to the bottom, or swimming slowly among weeds, reduces their ability to disperse freely throughout their potential range. The only significant seasonal migration undertaken by Perch

appears to be a winter one from shallower to deeper waters in deep lakes (Allen, 1935). Indeed the conservative behaviour of perch is such that only half a century ago, as Regan (1911) pointed out, perch were *"local and introduced"* in occurrence in Cornish rivers. Regan assigned to man a prominent role as distributional agent for perch, and in addition for carp, tench and pike.

This brief but relevant account of perch natural history indicates the 'patchiness' of their distribution, and the factors in their biology contributing to this. It is clear that perch are much less likely to populate rapidly and thoroughly the whole of their potential range than Salmonid species since the latter are much better swimmers and have in their catadromous phase the capacity to penetrate far into marine waters and hence to move along coastlines from one river mouth to another.

Finally it may be noted that the writer's own observations on P. fluviatilis in nature, during a number of years in Tasmania, have served to confirm that these principles of the perch's general biology also apply to this species in this Southern Hemisphere country.

Given this background, plus laboratory experience of the perch's temperature tolerance, it is possible to attempt an analysis of world distribution.

(ii) Critical regions in the distribution

It will be best to consider first a clear example of the distribution of perch in a country where the limits of its occurrence are known fairly accurately, yet where temperature is unlikely ever to exercise a limiting effect.

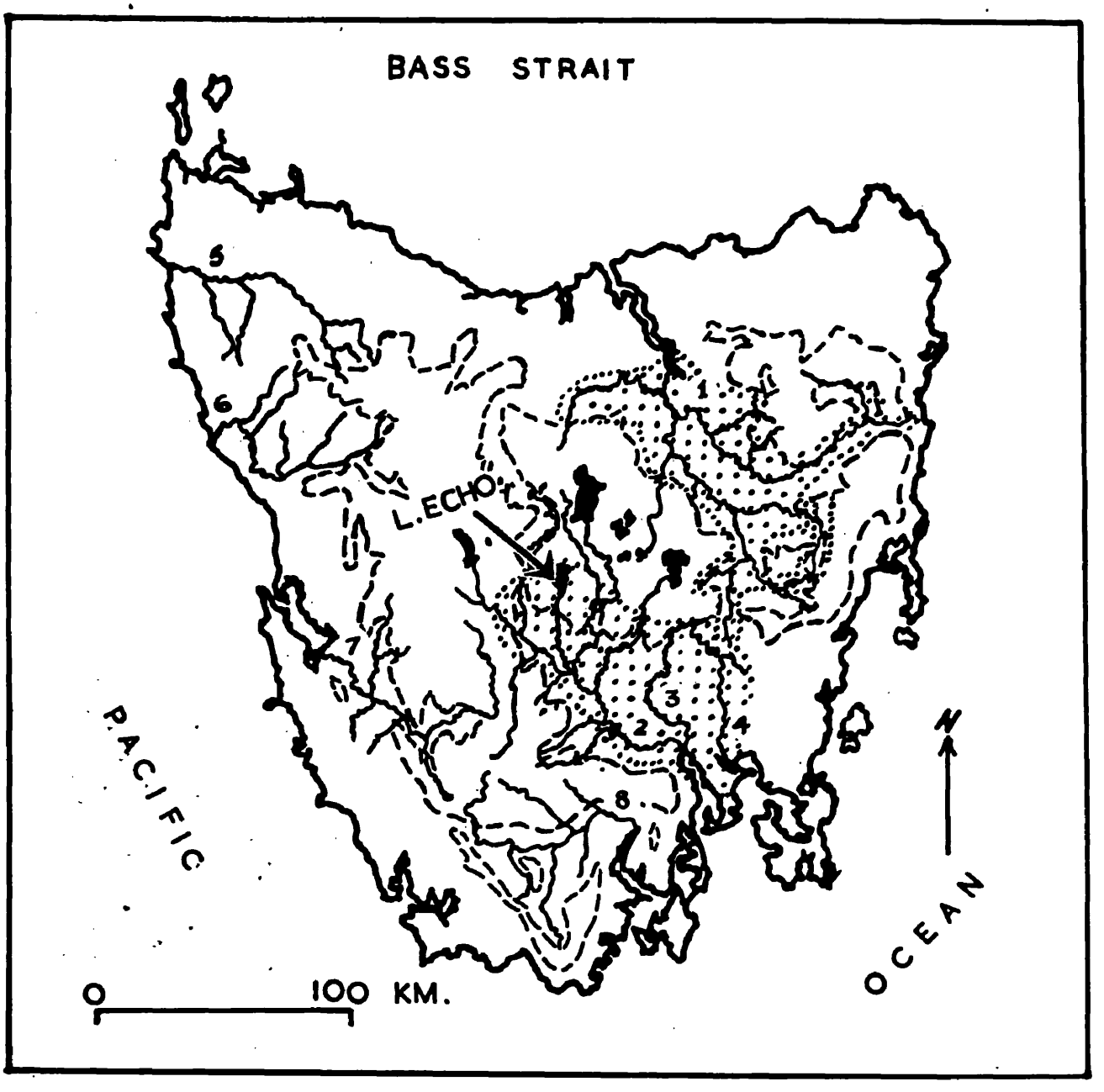
P. fluviatilis was first introduced into Tasmania from Britain in 1861. The species has flourished in this island State of Australia, but only in rivers in which still and weedy conditions prevail for at least a large part of their course. Their Tasmanian distribution is thus limited to the lower reaches of one river system in the north (Macquarie-South Esk River system) and to three systems in the south (Derwent River and its lowland tributaries, and the Jordan and Coal Rivers). In the many rivers with short, steep courses discharging into the sea along Tasmania's north-west coast, the perch is not found, or occurs only in a very few, suitable, isolated streams to which it has been locally introduced. It is apparently also absent from similar short, steep streams of the east and west coasts. There are three river systems of the west coast (Arthur, Pieman and Gordon), and one in the south (Huon) which all seem as if much of their courses would suit perch, yet appear to lack them.

Probably this is merely because these rivers are effectively separated from those containing perch and no attempt has been made to introduce them. The steep streams flowing from the Tasmanian Central Plateau lack perch and only one lake in this Plateau contains them. This is Lake Echo (Figure 6), and though the lower reaches of the River Dee which flows from Lake Echo contain perch they did not enter the lake originally from this river, since the lake was effectively isolated from river perch by the steep highland stretch of the River Dee itself. Perch were introduced into Lake Echo by the Brock family of Tasmania about 40 years ago. While they have flourished in the lake it should be noted that Lake Echo is mesotrophic in contrast to the oligotrophic lakes characteristic of the Tasmanian highlands, and has an extensive shallow littoral, with numerous large plant beds.

In Tasmania, then, the principal factors which limit perch distribution seem to be the higher land masses, which isolate one river system from another and produce so many steep highland streams both on the coasts and coming from the Central Plateau.

The action of very similar factors will explain present distribution of P. fluviatilis in New Zealand,

Figure 6. Present limits of distribution for Perca fluviatilis in Tasmania are shown stippled. In the north is the Macquarie- South Esk River system (1); in the south the Derwent River and its tributaries (2), and the Jordan (3) and Coal (4) Rivers. The Arthur (5), Pieman (6), Gordon (7) and Huon (8) Rivers are also shown. The highlands, from which perch are largely excluded, save for Lake Echo (marked with arrow), and which probably influence their absence from some coastal rivers (see text), are shown as areas enclosed by broken lines. These lines enclose regions 2000 ft. or more high.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to which the species was introduced about the same time as to Australia. Allen (1960, personal communication) says that *"They occur in a scattered fashion from the extreme south to the north Auckland Peninsula ..... They are .... found in shallow lakes and sometimes in the quieter reaches of rivers. I suspect that the very scattered distribution is due to the fact that they have not been introduced into many waters for which they would be potentially suitable."*

When Britain is considered (Figure 5) it is seen that perch are missing only from the Scottish Highlands, i.e. Scotland north of Loch Lomond, in Loch Lomond itself they are abundant. Actually perch do occur in a very scattered fashion in many parts of northern Scotland, but their distribution is probably due mainly to man, especially where they are occasionally found in hill lochs. They seem, however, to be absent from hill streams. The writer is indebted to members of the staff of the Freshwater Fisheries Laboratory, Pitlochry, for information on perch distribution in northern Scotland.

All over Britain the perch possibly owes its present ubiquity mainly to man, especially when its limited occurrence in Cornwall only 50 years ago is remembered. But again stream gradient seems to play a most important

part in imposing limits. Low temperature cannot be very important, as the species is found in rivers all over Eurasia within the Arctic circle (Berg, 1948-9 & Figure 5).

It should now be apparent that it is not enough to say with Darlington (1957) *"I think that climate has a .... profound effect on the distribution of ..... freshwater fishes, although its importance is not often recognised ....."* (though this statement may be, essentially, perfectly correct), because high land masses, resulting in streams with steep gradients may oppose effective dispersal of perch. Such factors might well produce a superficial appearance of the kind of *"zonation with climate"* that Darlington and others have shown for marine fish faunas of the world.

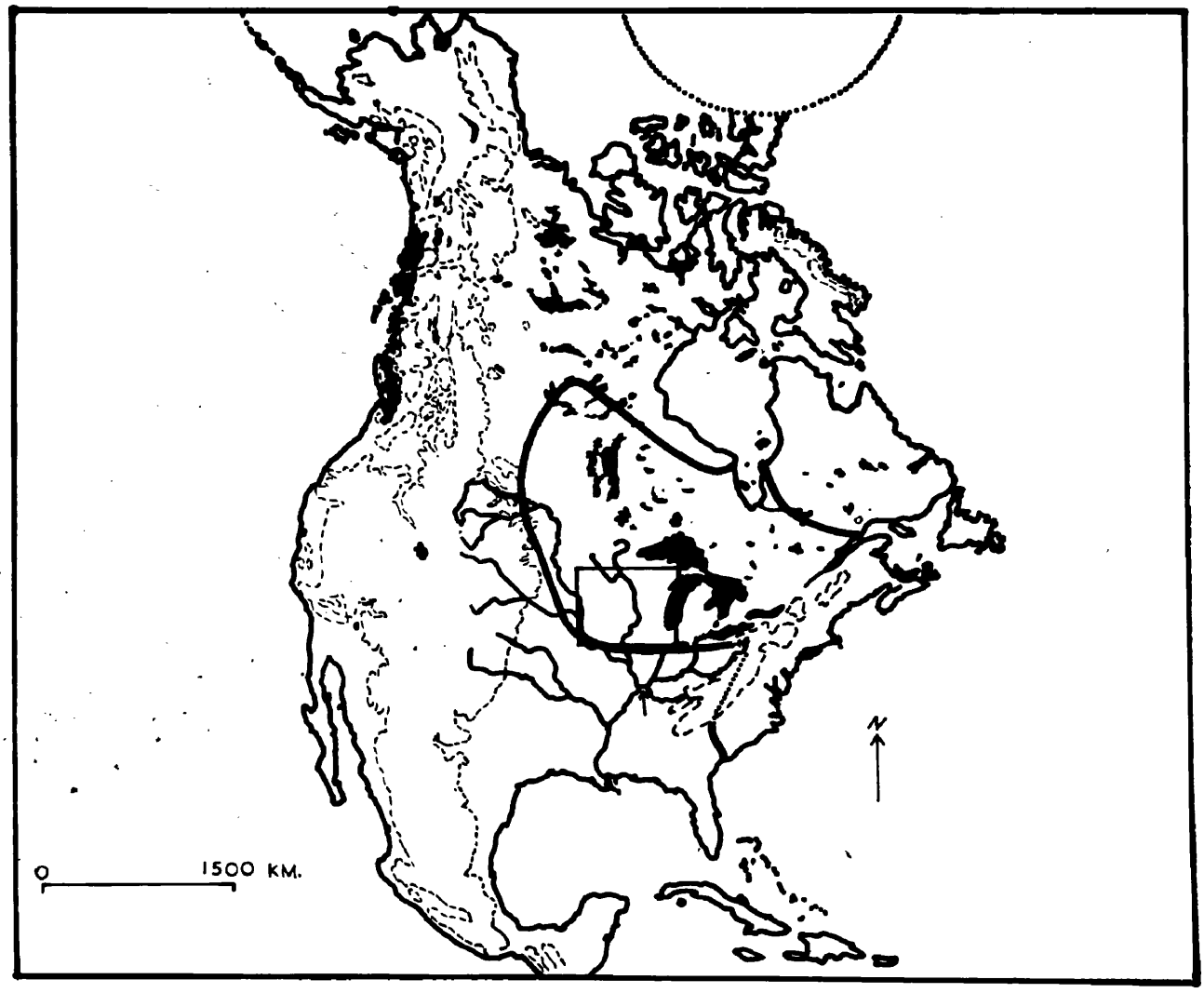
There appear to be only two obvious regions in the world, where Perca spp. are common, which are suitable for testing an hypothesis that higher water temperatures may limit the penetration of perch towards the equator. One of these is the Mississippi-Missouri-Ohio River system of the U.S.A., the other being the Murray-Darling River system of New South Wales. Perca spp. occur in each of these systems and their tributaries. The systems have certain features in common: They are very

extensive, covering a wide latitudinal range, from approximately 47° to 30° in the case of the American system, and 34° to 26° in the case of the Murray-Darling; they have only a very slight gradient over the major part of their courses, with a comparatively slow rate of flow and a sometimes wandering bed; large material obstacles likely to provide effective barriers to perch are lacking. Thus it seems probable that temperature will be the main physical characteristic likely to change in a graded manner with latitude along the course of these systems, though of course there may be local or periodic variations in such factors as oxygen values, levels of dissolved substances, flow rates, etc.

Figure 7 shows the present distribution of P. flavescens in America based on the most recent information the writer has been able to obtain. West of the Appalachian Mountains perch do not occur further south than the north-eastern corner of Kansas. To the east of this point the southern limit moves progressively, though slightly, further north through Missouri, Illinois, Indiana and Ohio, and in Ohio perch are found only rarely south of the centre of the State. East of the Appalachians perch appear in the large rivers running to the coast, and extend down into southern South Carolina. They have been

Figure 7. Limits of distribution of Perca flavescens within North America. The species is absent from the higher parts of the Appalachians, and the Rocky Mountains chain prevents it reaching the Pacific Coast. Along the Atlantic seaboard it occurs further south than in the Middle West. The massif of the Rockies to the west and the Appalachians to the east are indicated by broken lines. In the case of the Rockies the ground so indicated is at least 3000 ft. above sea level.

The small rectangle signifies the section of the Mississippi River depicted in Figure 8. The confluence of the Mississippi and Missouri Rivers is marked with an arrow.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released in several inland reservoirs and are doing well in at least one of these as far south as north Georgia (King, 1960, personal communication). Earlier authors (e.g. Jordan & Evermann, 1896) and recent ones (e.g. Trautman, 1957) are agreed that perch have always been practically absent from the Ohio River proper. Trautman (1957) writes that "*Kirtland .... in 1850 believed that the perch was moving southward from the newly constructed canals (from Lake Erie) and would eventually extend its migrations into the Ohio River; but later events proved that this southward extension progressed no further than central Ohio (Buckeye Lake). After 1900 many thousands of Perches were taken from Lake Erie and liberated in the streams of southern Ohio, but these introductions failed except for occasional strays in streams or establishment, often of dwarfed individuals, in some impoundments. During the 1920-32 fish survey a total of fewer than 20 Perches were captured in southern Ohio, despite the fact that I made a practice of seining in the vicinity of recent plantings.*" This interesting statement is reproduced here because it indicates clearly that nearly all perch failed to survive in water which at face value looked suitable for them. Furthermore, it is clear from the above references that perch are plentiful in the waters of northern Ohio, lakes in northern Indiana,

and in the tributaries of the upper Mississippi; Trautman mentions the upper waters of the Scioto River (a northern tributary of the Ohio River) and the Wabash, Illinois and Rock Rivers, which are all Mississippi tributaries .

Now for the purposes of this evaluation it is considered that the maximum water temperature of midsummer (usually July or August in the Northern Hemisphere, January or February in the Southern Hemisphere) are those which will be critical in controlling perch, if high temperatures are at all responsible.

In Buckeye Lake, central Ohio, there is a record of surface temperatures between 29 and 31°C. in late July - early August, 1930, though it is likely that lower temperatures may have prevailed over much of the lake, while it is also probable that higher temperatures occurred than those recorded (Tressler, Tiffany & Spencer, 1940). There seems also to have been a small but definite vertical temperature gradient in the lake, of the order of 7°C. So when the surface was, say, 30 - 32°C., it probably did not exceed 25°C. at the bottom (6.25 m.). Since perch can live for a prolonged period at 25°C., and since, anyway, Buckeye Lake does

contain an enduring perch population, this lake is mainly of interest in showing that shallow ponds, whose depths are not greater than a couple of metres, would be likely to exceed 30°C. in summer in central Ohio. Further south, still higher temperatures can be expected, and the critical level for perch is apparently reached in the Ohio River. Trautman (1960, personal communication) has added to the above extract from his book. He believes that high temperature is the principal factor responsible for the relative absence, or stunting, of perch in southern Ohio, stating that water temperatures in this part of the State are often between 26.7 and 31.7°C. for hours and days at a time. Though he finds that perch may tolerate temperatures in the field of about 33°C. for short intervals, they are absent from, or represented by stunted individuals in, waters where the summer temperature is frequently above 29°C. In view of the immense experience and authority of Trautman in the natural history, systematics and distribution of fresh-water fish, especially in Ohio, his views can be accorded great respect.

Galtsoff (1923 - 4) published a limnological survey of the upper Mississippi including a map and temperature

data for this river and some of its tributaries in midsummer over a stretch which is very important in this study. Figure 8 is a map based on his Figure 3 and shows that at all points surveyed from Fairport, Iowa, downstream to Keokuk, temperatures were in the range 27 to 33.3°C. The mean temperature of this section of the river was therefore about 30.5°C., but it would be the higher temperatures in this range (say 30 to 33.3°C.) which would be most critical for perch.

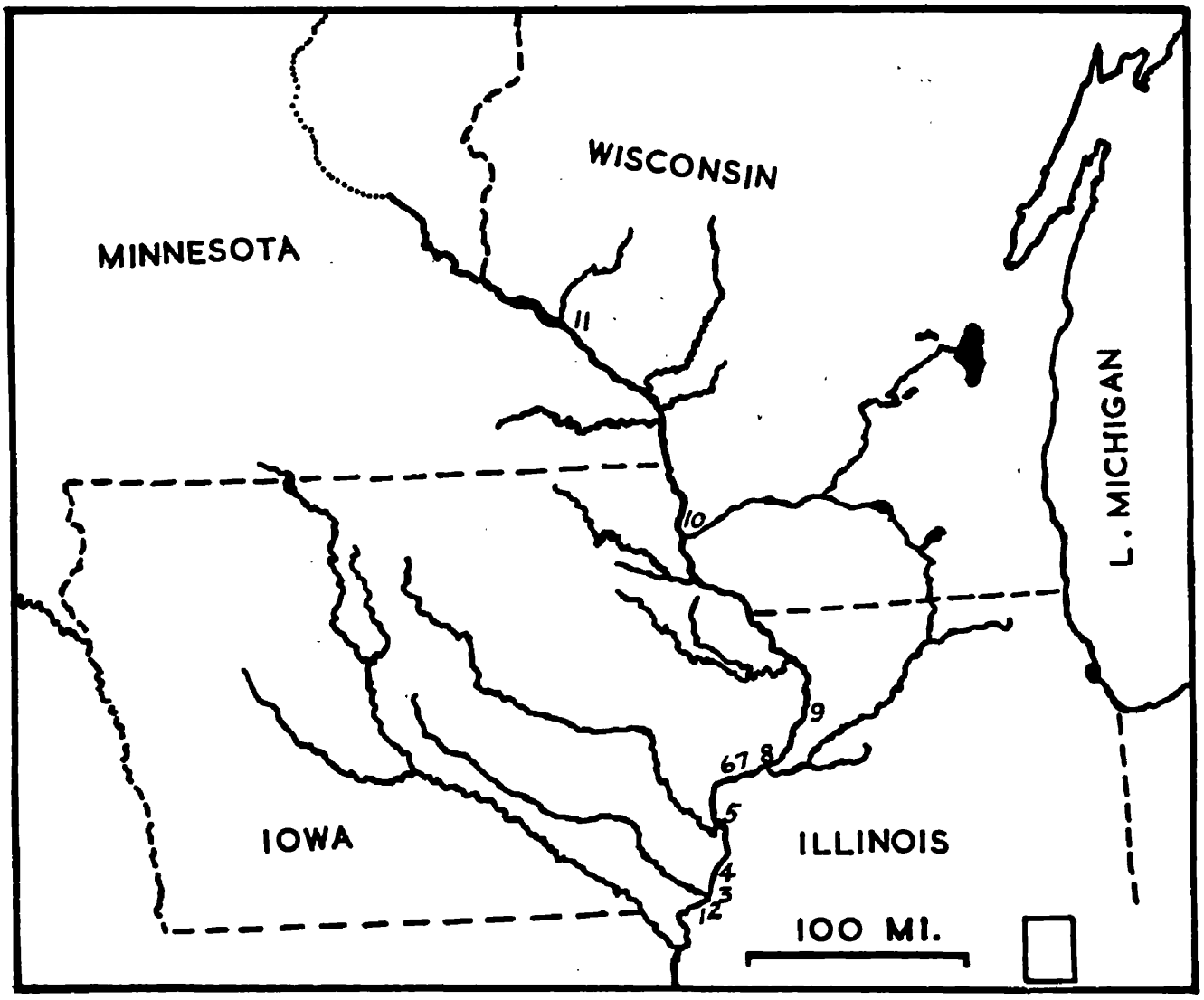
The latitude of this part of the Mississippi, from Fairport to Keokuk, is very nearly the same as for central Ohio. Indeed, there is only about 1° of latitude difference between central Ohio and the northeast corner of Kansas which, as mentioned earlier, is the southernmost limit for perch in central North America. It should be noted that the Ohio River enters the Mississippi far below the most southern occurrence of perch in the latter or its tributaries.

However, the data of Galtsoff fail to tell the whole story. Platner (1946) collected temperature data from the same section of the Mississippi during 1944. The highest midsummer temperature he recorded was 28.5°C. (August), for a site just above the middle of Iowa during low water. Further south temperatures did not even

Figure 8. Temperatures in the Mississippi River in July-August, 1921. Values and map based on Galtsoff (1923-4). For further explanation see text.

| Map position | Place | Date | Temp. (°C). |
|---|---|---|---|
| 1 | L. Keokuk near Dallas City, Ill. | 15/7/21 | 30.4 - 33.3 |
| 2 | Slough of Skunk R. above Dallas City | 20/7/21 | 28.5 - 29.4 |
| 3 | L. Keokuk 4-5 mi. above Dallas City | 20/7/21 | 27.0 - 31.1 |
| 4 | Mississippi R. 1 mi. above Burlington, Ill. | 14/7/21 | 31.0 |
| 5 | All in Mississippi R. or mouths of tributaries near N. Boston, Ill. | 13/7/21 | 31.0 - 33.3 |
| 6 | All in Mississippi R. or mouths of tributaries at or near Fairport, Iowa | 11-12/7/21 | 30.2 - 31.1 |
| 7 | Mississippi R. near Fairport | 9/8/21 | 25.5 - 25.6 |
| 8 | Mississippi R. above Davenport | 11/8/21 | 25.6 |
| 9 | Mississippi R. 6 mi. above Clinton | 12/8/21 | 25.0 |
| 10 | Mississippi R. above Prairie du Chien | 15/8/21 | 23.3 |
| 11 | Mississippi R. 1 mi. above Wabash | 18/8/21 | 21.7 |

The small rectangle in the lower right corner signifies the area and location of Champaign County. The streams of this region were surveyed, and their fish populations censused, by Thompson & Hunt (1930-2). See text for further information.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reach this value. Certainly perch are abundant in both the Mississippi and Missouri Rivers where they border Iowa (Trautman, 1960, personal communication). Thus it seems best to suggest that though the very high temperatures Galtsoff recorded for the summer of 1921 may not occur every year, yet the fact that they do occur, and that perch are very rare below Iowa, seems to imply that temperature is limiting their penetration further south in this system.

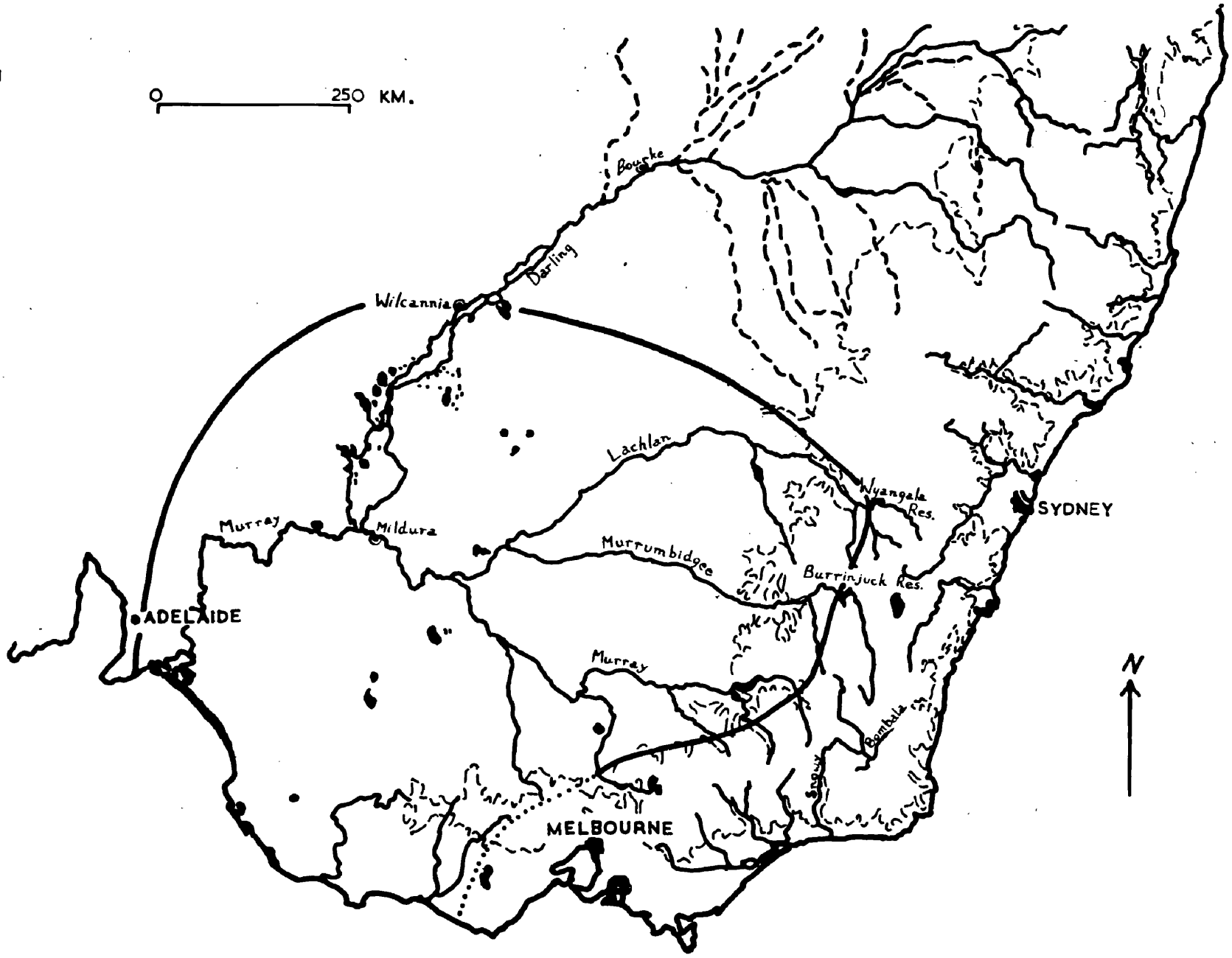
Also indicated in Figure 8 is Champaign County, an area of stream systems in Illinois whose fish populations were extensively censused by Thompson & Hunt (1930 - 2). These streams eventually discharge into the Mississippi River, either directly or via the Illinois or Ohio Rivers. The streams do not contain P. flavescens, though there is a long list of other fish species, and from the descriptions of the streams by the authors they would appear to have many stretches suitable for perch. There are, however, two probable reasons, both connected with high temperature, why perch are lacking. Firstly the confluence of these streams with the Mississippi, whether through the Illinois or Ohio Rivers, or directly, are below the lower fringe of distribution of P. flavescens (Figure 8). In the second place Thompson & Hunt state that temperatures

in the streams may exceed 32°C. in unshaded sections in midsummer. So the streams both enter the Mississippi further south than the limit of what has been considered thermally tolerable for perch and contain, within themselves, stretches where temperatures may reach or exceed this limit.

It is well-known that the Mississippi-Missouri system is extremely turbid (especially the Missouri; Platner, 1946, provides values), and in case it be thought that perch might be limited by the effects of silt and turbidity it is worth pointing out that Trautman (1960, personal communication) believes this to be only of minor importance. He has indicated that perch in Ohio frequent turbid waters and that *"Sandusky Bay, an embayment of Lake Erie in Ohio, has turbid waters with much silt in suspension, especially in the spring, yet perch spawn abundantly in Sandusky Bay"*. The present writer has also seen actively growing and reproducing perch populations in farm dams in Tasmania in which the water is very turbid (turbidity several hundred p.p.m.). It is only where great turbidity or silting may destroy plant beds that perch are likely to be affected.

The distributional limits of P. fluviatilis in Victoria and New South Wales are indicated in Figure 9. Though not very common in the Darling River they occur, but

Figure 9. Limits of distribution of Perca fluviatilis in the Murray-Darling system of New South Wales & Victoria shown as accurately as can be determined at present (heavy line). The Australian Alps & the Great Dividing Range are indicated by the area enclosed by broken lines, and which represents land over 1000 ft above sea level. See text for temperature data.


0
250 KM.
Bourke
Darling
Wilcannia
Lachlan
Wyangala Res.
SYDNEY
Murray
Mildura
Murrumbidgee
Burrinjuck Res.
ADELAIDE
Murray
Bombala
MELBOURNE
N

apparently not further north than Wilcannia (Lake, 1959). Maximal temperatures in the Darling at both Wilcannia and Bourke appear to be 29°C. in a normal summer, with but little decrease with depth. Perch are common in the Lachlan, Murrumbidgee and Murray Rivers, being blocked in the former two by the Wyangala and Burrinjuck Reservoirs, or by the hill streams which flow into them: it is not certain which. The Murray is usually about 3°C. cooler than the Darling in summer, but in the lower Lachlan, during a protracted heat wave in the summer of 1958-59, temperatures reached 32°C. Dead perch were seen here and others, gill-netted, died soon afterwards (Lake, 1960, personal communication). Downstream near Mildura the temperature in the Murray-Darling system rarely exceeds 26.5°C.

Thus it begins to appear likely that 29 to 32°C. is the approximate range for higher temperatures which may control distribution of Perca spp. towards the equator, especially when field experience is considered in conjunction with laboratory data. Of course, the actual temperature imposing a thermal limit will vary somewhat with the thermal history of the fish, the duration of its exposure to the highest temperature encountered, and other factors such as oxygen and carbon dioxide tensions, etc.

In applying the principles which appear to govern perch distribution to explain the present limits of the world range of perch several points must be born in mind. There may be numerous waters outside the present range where perch will eventually flourish, through man's activities. For even when the surface temperatures of such waters exceed the lethal level for perch the profundal of deep meso- or oligotrophic lakes may be cool enough, and have sufficient oxygen reserves, to permit perch to survive a difficult summer. Alternatively, where the altitude of a lake is sufficient to offset the effects of low latitude (nearness to equator) perch could survive. But here they would certainly need to be introduced by man, since the barrier of height (i.e. stream gradient) would usually prove impassable. A typical example of this kind is afforded by the lakes of the Sierra Nevada of California. These are far to the west of the range of perch in America, and further south than the lowest limit of their distribution, yet because of their high altitude the summer temperatures in these lakes do not exceed about 15°C. (Reimers, Maciolek & Pister, 1955).

However, it is in rivers of a certain kind, and in shallow ponds, where we may expect to witness high

temperatures most clearly exercising their influence. Though it obviously cannot be expected that data will be available for temperatures in the innumerable ponds at the same or lower latitudes than the limits of perch distribution, it may be that water temperatures will, often enough, approximate to prevailing air temperatures in midsummer to enable a reasonable prediction of the one to be made from the other. In Figure 10 maximum monthly water temperatures in the top metre of two Tasmanian farm dams are plotted against the mean monthly maximum air temperature for Hobart,* the air temperature data having been collected at a station only a few miles distant from the dams and at much the same altitude (station at 177 ft.). These dams have been described limnologically in connexion with fishery investigations (Weatherley, 1958). The correspondence of water and air temperatures is apparent, though it is not to be inferred that water temperatures are causally related to air temperatures. The line in Figure 10 has the slope which

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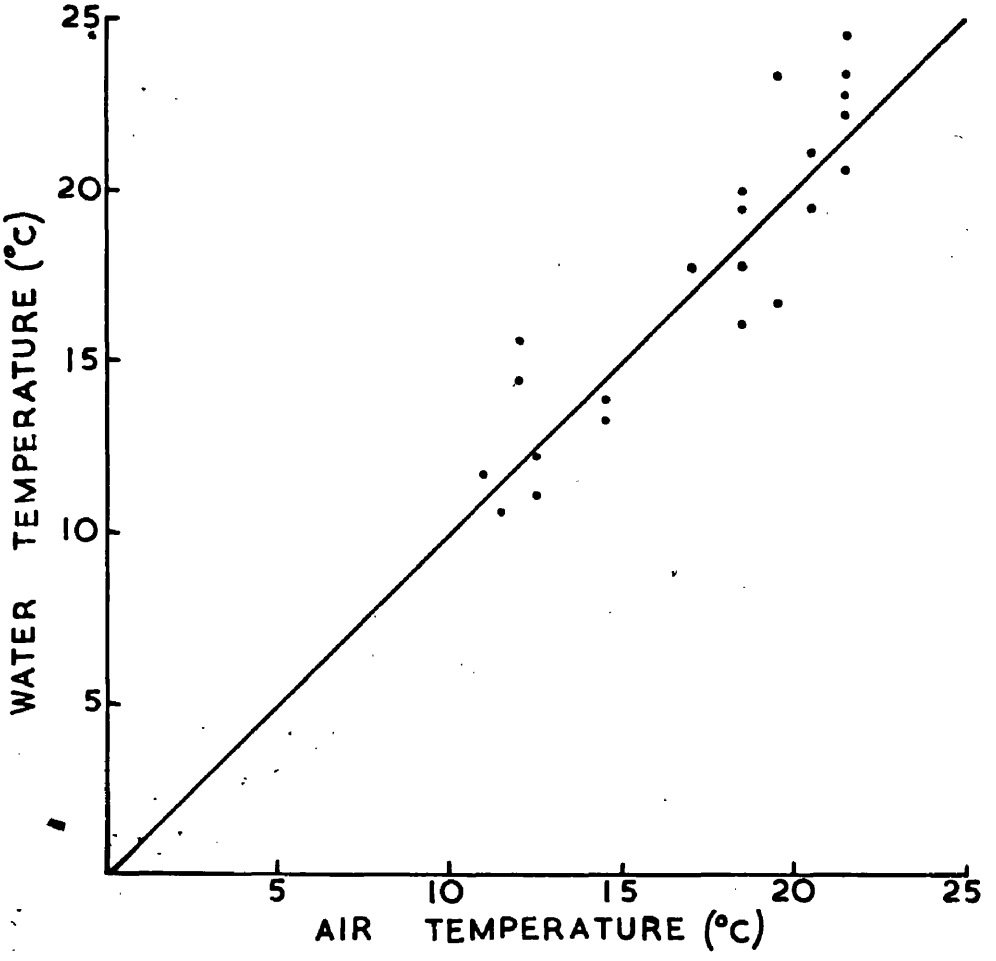
* *These and all other air temperature data in this study were obtained from Tables of Temperature, Relative Humidity and Precipitation for the World, compiled by the Meteorological Office of the Air Ministry, and published by H.M. Stationery Office, 1958.*

Figure 10. Surface water temperature versus corresponding mean monthly maximum air temperature for two small Tasmanian farm dams.


25
20
15
10
5
WATER TEMPERATURE (°C)
5
10
15
20
25
AIR TEMPERATURE (°C)

would represent a one-to-one ratio between air and water temperature. As is obvious, it serves as a good regression line for these data. Thus air temperature is likely to be very useful in predicting approximate water temperature in shallow, easily-mixed water bodies near sea level.

It appears also that the temperatures in slow-flowing rivers may often correspond to maximum air temperatures, especially in midsummer, and when the water is sufficiently far from hills or mountains from which cooler water may flow. The mean maximum air temperature for July for stations near the critical section of the Mississippi River, covered in the report of Galtsoff (1923-4), are arranged in Table 4.

In Buckeye Lake, central Ohio, the surface temperature maxima are very close to the mean maximum air temperature at nearby Columbus.

Thus it appears that it may be practicable to infer water temperatures from known air temperature maxima in summer in those regions where it seems possible that perch may be encountering a thermal barrier.

*Table 4.*

*Correspondence between air and water temperatures in midsummer (°C).*

| Station | Mean max. air temp. (July) | Mean water temp. (July) | Mean range in water temp. |
|---|---|---|---|
| Des Moines | 30.5 | | |
| Lincoln | 31.5 | | |
| Kansas City | 31.5 | | |
| Sioux City | 30.5 | | |
| Minneapolis | 28.0 | | |
| St. Louis | 31.0 | | |
| Mississippi R. (L. Keokuk to Fairport) | | 30.6 | 27.1 - 33.3 |
| Columbus (Ohio) | 29.5 | | |
| Buckeye Lake | | 30 (not more than 25 at depth) | possibly up to 32 |

(iii) World-wide distribution of perch: a detailed analysis

This will now be attempted on a country-by-country basis.

America

Figure 6 shows the limits of P. flavescens in North America. Perch are not found in the north. This may be because this is largely a region of ground permafrost where the lakes are often frozen for a great part of the year, and where general thermal conditions are too low to permit survival. To the west perch are turned back by the steep stream gradients associated with the huge massif of the Rocky Mountains, and are also absent from the North American Pacific Coast, both of Canada and the U.S.A. Because of the moderating influence of the Pacific Ocean, air temperatures at coastal stations even in California are quite appreciably lower in midsummer than those at similar latitudes in the Middle West of the United States, but the Rockies have effectively prevented perch from entering this region. However, it remains doubtful whether perch could become widely established in the Pacific Coast rivers, even if they could gain access to them or were massively introduced, since most of them are short and swift, descending rapidly from the

Rockies into the Pacific.

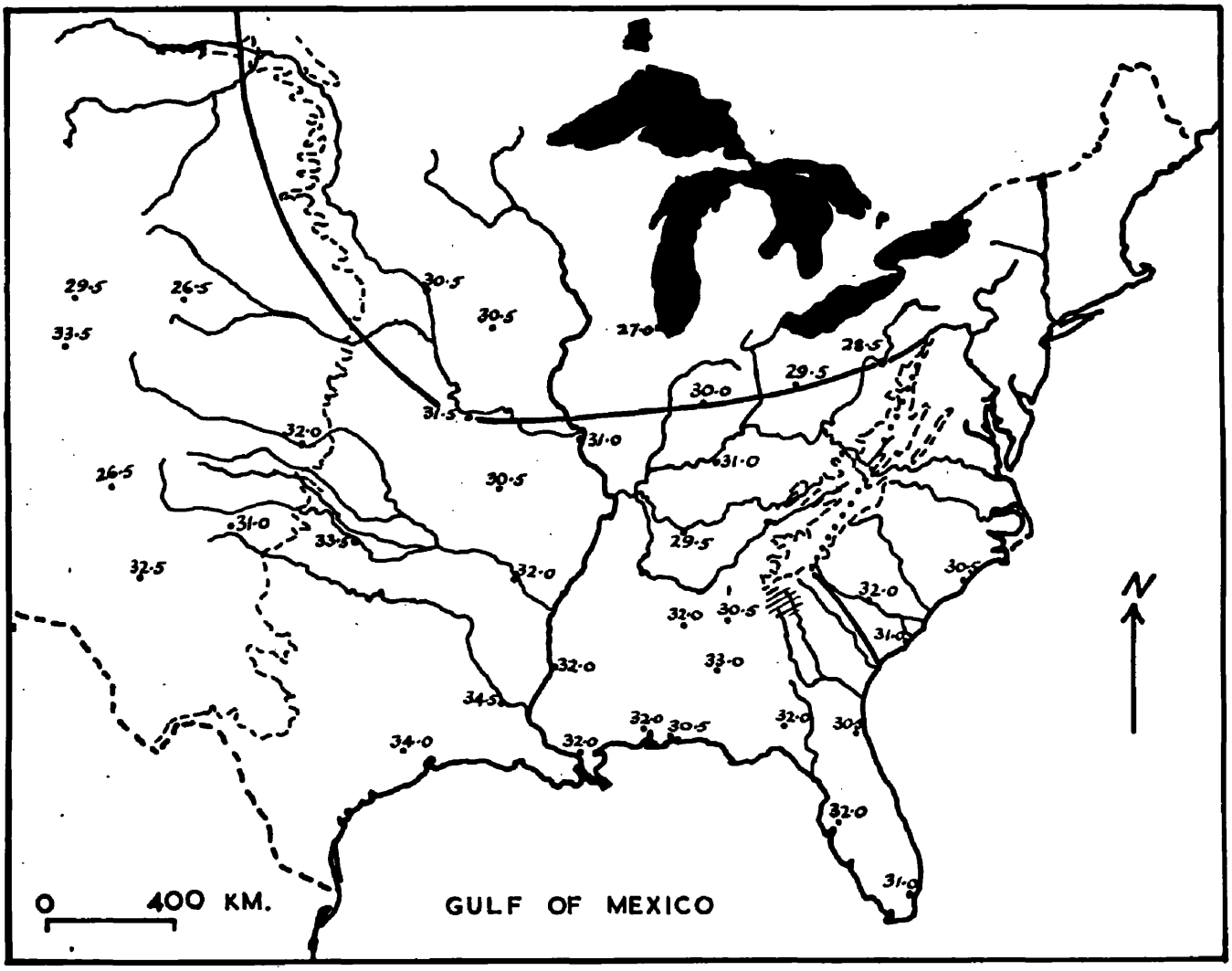
In considering limits of perch distribution in the Middle West of the U.S.A. Figure 11 should be taken into account. It is apparent that west of the Appalachians the southern fringe of perch occurrence is, in general, associated with air temperatures of 30-31°C. Where temperatures exceed this level, as they do in a southward direction right down to the Gulf of Mexico, perch are lacking. On the eastern side of the Applachians numerous rivers run into the Atlantic and here perch are found down to southern South Carolina (Figure 11). They thus extend further south on the east coast than in the Middle West, probably because of the moderating influence of the Atlantic Ocean (a view, incidentally, that is shared by Trautman, 1960, personal communication). In addition these east coast rivers with their relatively short yet largely lowland courses arise in the Appalachians where somewhat cooler conditions prevail.

Perch have survived in at least one north Georgia reservoir (Figure 11), though from the map it looks as if they would encounter a rather abrupt thermal barrier in lower Georgia.

Western Europe

In Western Europe (Figure 5) only northern Scotland,

Figure 11. Southern distributional limits of Perca flavescens in North America in relation to mean maximal air temperatures of midsummer. Location where perch are surviving in North Georgia indicated by hatching. See text for further explanation.


29·5
26·5
33·5
30·5
30·5
27·0
28·5
29·5
30·0
31·5
31·0
31·0
32·0
26·5
30·5
31·0
33·5
29·5
32·5
32·0
30·5
32·0
30·5
31·0
32·0
33·0
34·5
32·0
30·5
32·0
34·0
32·0
32·0
31·0
0 400 KM.
GULF OF MEXICO
N

the greater part of Norway, Spain, most of Italy and much of Greece lack perch P. fluviatilis, and as Britain has already been considered the survey may start with Norway.

Norway: It seems fairly certain that much of Norway lacks perch because the few rivers in the northern half of the country are so short and steep, arising as they do in the Kjølen Mountains and descending rapidly to the sea. In addition, much of this part of the country is within the Arctic circle, and since it is also high must be extremely cold. Only in south-eastern Norway are there numerous lakes and some rivers with fairly extensive lowland reaches where perch might find suitable habitats, and these waters do contain them (Figures 5 & 13).

Spain: Perch are absent from the Iberian Peninsula, evidently being excluded by the Pyrenees. Temperature is apparently not involved since air temperatures to the north of the Pyrenees (i.e. of the south of France), from Bayonne to Nice, do not exceed 28°C., while the waters descending from the high altitudes of the Pyrenees will be cooler than the bigger river systems of southern France. Indeed air temperatures do not become unduly high until nearly halfway down the Peninsula; the mean maximum air temperature does not touch 31°C. until Madrid is

reached. So temperature is unlikely to have barred perch at least from northern Spain.

On the other hand, though much of Spain is high, because it is a plateau extensive rivers exist, with wandering courses of slight gradient, and there seems no obvious reason why perch could not live at least in the more northern of these if they could cross the formidable barrier of the Pyrenees in sufficiently large numbers.

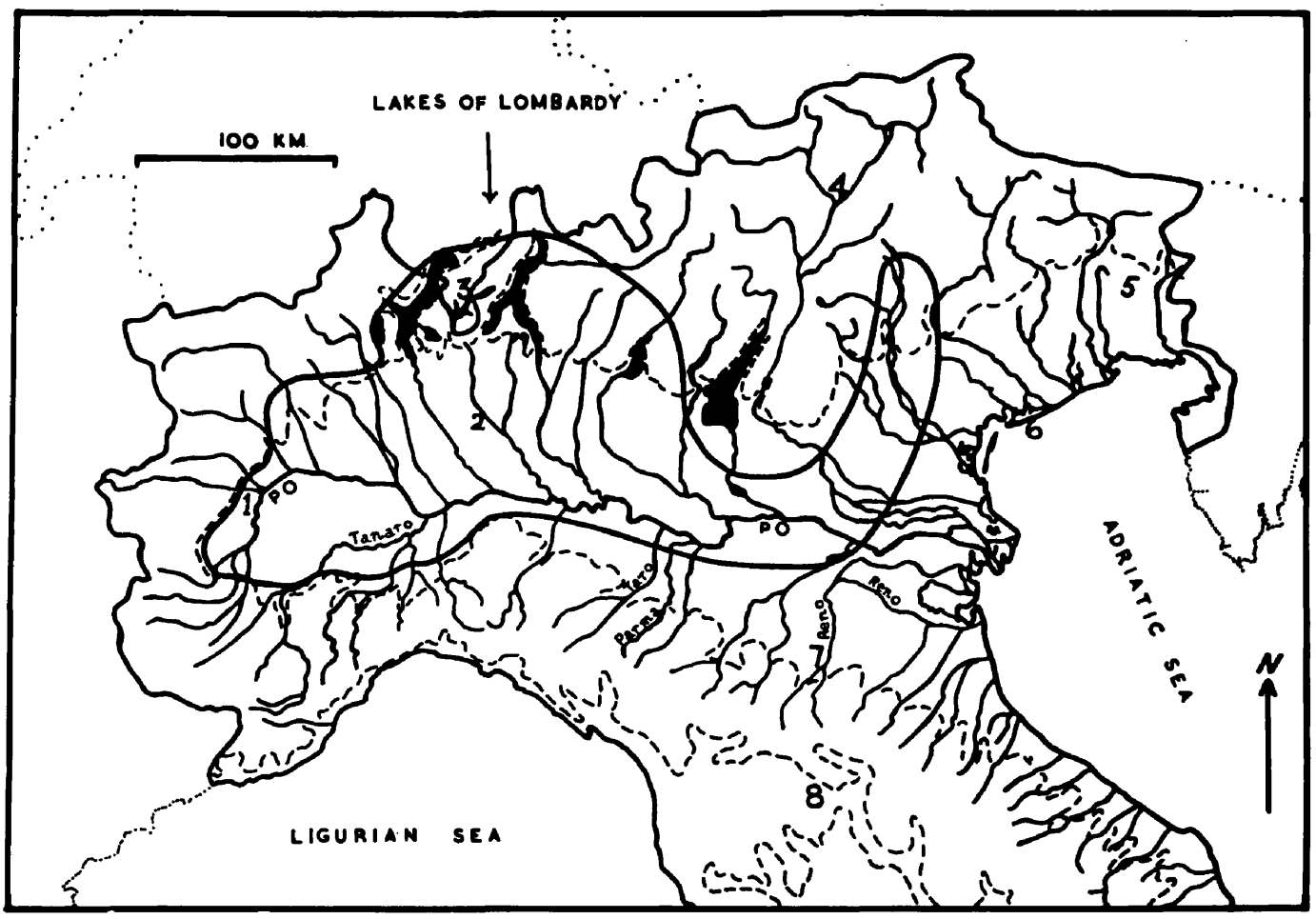
Italy: Italy furnishes one of the more interesting examples of perch distribution in Europe. Figure 12 shows the limits in Italy today, based mainly on the information of D'Ancona (1960, personal communication). Perch are not found in the waters of the high Alps, and their limited occurrence in Italy, restricted as it is to the highland lakes of Lombardy and to the Po Valley, is likely to be the result of introduction by man, since it is almost certain that the Alps would have been impassable.

Though perch are present in the major part of the Po River and most of its northern tributaries, many of which flow from the lakes of Lombardy, such as Lakes Maggiore, Lugano and Como, they do not inhabit the highland reaches of these tributaries, even though many of the tributaries come directly from lakes which do contain perch (D'Ancona,

Figure 12. Distributional limits of Perca fluviatilis within the Po basin of north Italy; limits signified by heavy line. The high lands of the Alps and Appenines are enclosed by broken lines, areas within them representing country 1000 ft. and more above sea level. Numerals correspond to cities for which the mean maximal air temperatures of midsummer are given below.

| No. | City | Air temp. (°C.) |
|---|---|---|
| 1 | Turin | 29.5 |
| 2 | Milan | 29.5 |
| 3 | Lugano | 28 |
| 4 | Bolzano | 29 |
| 5 | Udine | 28 |
| 6 | Venice | 27.5 |
| 7 | Bologna | 31 |
| 8 | Florence | 31.5 |
| 9 | Ancona | 27.5 |

See text for additional information.


LAKES OF LOMBARDY
100 KM
1
2
3
4
5
6
8
PO
Tanaro
PO
Taro
Parma
Arno
Reno
ADRIATIC SEA
N
LIGURIAN SEA

1960, personal communication). This is apparently a further very clear demonstration of the effect of unsuitable gradient conditions on perch distribution even within rivers containing them.

Of southern tributaries to the Po only the Tanaro, Taro and Parma Rivers carry perch. This is curious, though these rivers seem to be among the few southern tributaries with extensive lowland reaches. In the southern tributaries to the upper Po perch are probably turned back by the Appenines.

The apparent absence of perch from the River Reno which traverses the southern part of the Po Plain is interesting. It is possibly a temperature effect, since the air temperature at nearby Bologna is 31°C., close to what is considered critical, and the Reno is mainly a slow, lowland stream, presenting good opportunity for equilibration between air and water temperature. Indeed, Bologna is probably the hottest locality in the Po Valley.

The streams running into the Adriatic from the Appenines do not carry perch, though in the north this is probably due to unfavourable gradients, since Ancona on this part of the coast is not unduly hot (Figure 12).

Now since perch have, by whatever means, managed to cross the Alps the difficulties of reaching other parts of Italy would seem relatively slight. Yet the remainder of

the country is evidently unsuitable on either, or both, of two counts. The "spinal" chain of the Appenines means that the majority of Italian rivers are short and swift, while the few that are otherwise, e.g. near Rome, Naples, Foggia and Taranto (in the south) are likely to be at or near the thermal limit for perch, since the respective mean maximum air temperatures for these localities in midsummer are 31°C., 30°C., 34°C., and 31.5°C.

Greece: Perch are plentiful in Doiran Lake on the Graeco-Yugoslavian border. They extend into Greece as far south as Thessally. Air temperatures exceed 30°C. in midsummer all around the Greek coast, but where rivers of an apparently suitable type exist, in northern Greece, perch are present. These northern rivers flow from the highlands of Bulgaria, Yugoslavia and of Greece itself. In Thessally the air temperature in midsummer reaches 34°C. (at Larisa, on the largely lowland River Pinios). Slightly cooler water from the Pindus Mountains in the west where the Pinios rises, probably keep the lower reaches of the river just below the thermal limit.

Eurasia and the Middle East

Turkey: Though perch are found in Bulgaria, where air temperatures do not exceed 30.5°C. and mostly are below

30°C. and there are several suitable rivers, Turkey lacks perch as do the lands to its south. Turkey is, of course, separated from Europe by the Black Sea. This separation is very slight at the Bosphorus, though it still represents a few miles of salt water which may prove a very difficult barrier for perch to bridge. However, most of Turkey is topographically unsuitable, being mountainous, and though the major part of the country partakes of the nature of an uneven plateau, in which certain localities have moderate air temperatures, these are usually very elevated (e.g. Erzerum, 6402 ft., mean maximum air temperature 26.5°C.; Sivas, 3888 ft., 27.5°C.).

On the Black Sea Coast temperatures are moderate, but there the rivers drop fairly steeply into the sea from high altitudes. Otherwise, especially in the south, midsummer, mean maximum, air temperatures are very high, e.g. Urfa 38.5°C. near the Syrian border; Izmir 33°C. and Bursa 31°C. on the west (Mediterranean) coast, where, in addition the few shortish lowland rivers are to be found.

U.S.S.R.: The most extensive and continuous occurrence of P. fluviatilis in the world is found in the territories of the Soviet Union. In the north they extend along the margin of the Arctic Ocean from the Kolar Peninsula in the west to the Kolyma River which is not far from the eastern tip of the U.S.S.R. Berg (1948-49) gave a general account

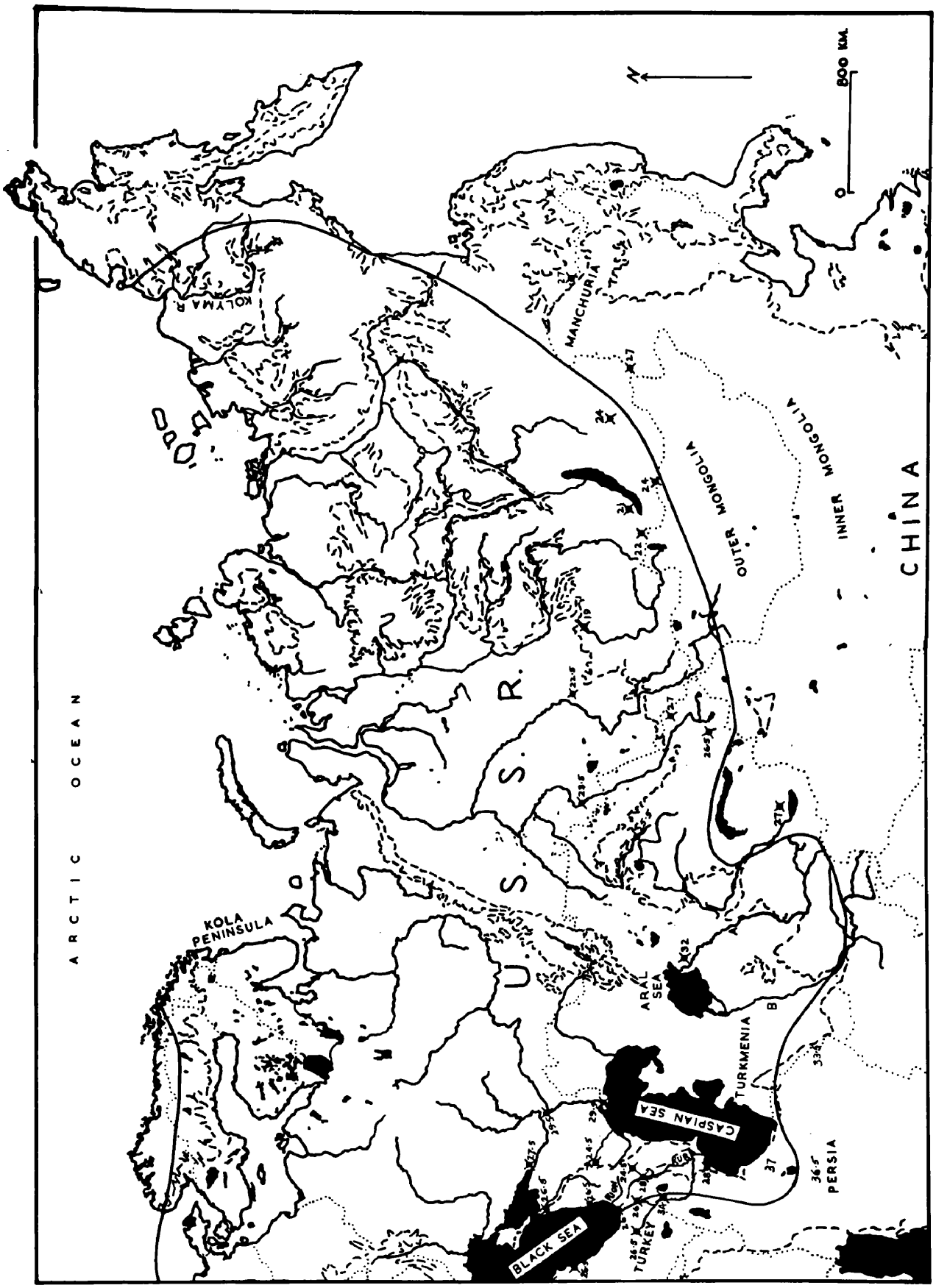
of their distribution, and Figure 13 is based mainly on his map of their distribution in Eurasia. It may be broadly stated that most of the major water bodies, especially river systems, of the U.S.S.R. carry perch. If they lack them there is usually no obvious reason for this, except perhaps isolation from other perch-containing drainage systems.

But though perch inhabit the southern reaches of the huge mature rivers which dissect the northern edge of the vast, high massif which culminates in the Himalayas, and separates the U.S.S.R. from China, they do not appear to extend much above 3000 ft., save perhaps in some lakes. Evidently they are not found much in Mongolia, in which the few existing rivers come from great heights and some are completely inland systems.

One area in Russia has sufficiently high air temperatures in midsummer to throw doubt on its ability to carry perch. This is the low country of Turkmenistan and Uzbekistan at the south and south-east of the Aral Sea. Here air temperatures are as high as those associated with perch limits elsewhere (Figure 13). Yet perch are certainly present in the Syr Darya, the Amu Darya and in the littoral of the Aral Sea (Berg, 1948-9, 1950). It is

Figure 13. Limits of distribution of Perca fluviatilis mainly within the U.S.S.R. (heavy line). Mean maximum air temperature of mid-summer for various places more or less close to the southernmost edge of this distribution are figured; they are generally less than the values associated with thermal limits; but see text in connexion with perch in Persia. Areas indicated by broken lines are all 1000 ft. or more above sea level; much of this is below 3000 ft. and especially in the north-east apparently contains perch waters. But the species has evidently been unable to penetrate above 3000 ft., and this very high land with its steep stream gradients governs southwards spread of the species.

A, Syr Darya; B, Amu Darya.


N
800 KM
0
ARCTIC OCEAN
U.S.S.R.
CHINA
MANCHURIA
OUTER MONGOLIA
INNER MONGOLIA
KOLYMA R.
KOLA PENINSULA
ARAL SEA
CASPIAN SEA
TURKMENIA
PERSIA
BLACK SEA
TURKEY

quite probable that the rather low relative humidity of this region accelerates heat loss from water through evaporation, thus keeping water temperatures rather lower than might be expected from the high air temperatures (see also section on Australia). Indeed Berg (1950) does state that rates of evaporation in this area are extremely high.

Perch also occur in the Caucasus region, in the Terek and Kuma Rivers. Here temperatures are not unduly high and the same applies to the Transcaucasian Rivers Rion and Kura, emptying into the Black and Caspian Seas, respectively, though it is interesting to note how completely the latter two rivers, in their valleys, appear to be isolated from other major systems.

Persia: Berg (1948-49) indicated that perch occur in northern Persia around and beyond the southern edge of the Caspian Sea. It is very difficult to understand how they would have become established in these highlands where no suitable river types seem to exist. Air temperatures are very high, too (Figure 13), but in northern Persia, as around the Aral Sea, relative humidity is uncommonly low. So if Berg (1948-49) is correct about the presence of perch here it is possible

that they can survive because water temperatures are lower than might appear from air temperatures.

South Africa: Perch have been introduced into South African waters, but the information it was possible to obtain on their distribution was too slight and fragmentary to add anything of interest to the present survey.

Australasia: The Victorian, New South Wales and Tasmanian distributions of perch (Figures 5, 6 and 9), together with that of New Zealand, formed part of the basis of this study, but the New South Wales distribution bears several other features which merit closer examination.

In the first place conditions are very dry in most of inland New South Wales, which is separated from the narrow strip of the east coast by the Great Dividing Range. Low humidity over most of this inland region probably helps to explain the fact that while water temperatures of the Darling River at Wilcannia and Bourke rise as high as 29°C., mean maximum air temperatures at these places in midsummer reach 35 and 38°C., respectively.

The absence of perch from coastal streams of New South Wales is probably due simply to their not having been introduced into them. For much of their courses these streams cross the relatively narrow coastal strip, but

temperatures cannot be too high here, for even as far north as Port Macquarie (some 180 miles north of Sydney) the air temperature has a mean midsummer maximum of only 28°C. Furthermore, these coastal streams arise in the relatively high, cool conditions of the Dividing Range. Rochford (1952, 1953a,b) has published temperature data on many of these streams which make it clear that most of them would be thermally tolerable for perch.

Lake (1960, personal communication) has noted an additional point of interest here. Perch *"occur in the Bombala River, a tributary of the Snowy River, but have not penetrated into the Snowy River itself .... The Bombala is a rather sluggish stream compared with the Snowy....."* (Figure 9). Here, again, is presumably seen the inability of perch to live in lively waters, even though they may inhabit other more suitable parts of the same river system.

(c) Discussion

The kind of correspondence between maximum air and water temperatures that has been suggested, and used to evaluate the influence of the latter in governing perch distribution, has a number of imperfections. Some of these are brought about as follows: by the effects of uncommonly low humidity, probably day length, excessive

turbidity, diel temperature range, the fact that water at the point of interest may be coming from a cooler highland source, or a warmer lake. Nevertheless, where air temperatures have been employed to infer water temperatures caution has been exercised in whatever ways were possible in the interpretation, and the possible effects of factors which might spoil correspondence were considered. Relative humidity has been especially noted for certain relevant regions, where it is conspicuously low (i.e. New South Wales, Persia south of the Caspian and the area to the south of the Aral Sea). In other places, such as central North America, the Po Valley, and certain other regions where temperature may be important, relative humidities have usually been moderate; i.e. something of the order of 60-80%.

If close similarity between the thermal responses of P. fluviatilis and P. flavescens be assumed several generalizations may be advanced to account for world distribution patterns of perch. In many instances the limits imposed may be attributed to the absence of suitable habitats in highland streams with their steep gradients. So perch will usually be turned back by high hills or mountains. These may also form a very effective barrier to their spread into waters which might in themselves be suitable (e.g. lakes in the Sierra Nevadas).

In addition we will not find perch in streams with short, steep courses (e.g. in much of Tasmania, on the North American Pacific Coast, much of Scotland and Italy, etc.). Streams with steep grades are obstacles to perch diffusion in two ways: The water will flow too rapidly for the fish to live comfortably, and such swift and turbulent streams will lack plant beds which perch need in which to deposit their ova. Thus, topographically unsuitable streams play an extremely important part in determining world patterns of distribution in these species.

The direct role of high temperature is probably only seen on a large scale in New South Wales and in the American Middle West, in very large river systems, where no extensive material barrier is apparent, and perhaps on a lesser scale in parts of Italy and Greece. However, if the range for limiting temperatures may be accepted as between 29-32°C., then even if the highland barriers preventing perch from reaching tropical latitudes in Eurasia could be overcome, temperature would still operate eventually to limit their spread towards the equator. On the other hand, perch could almost certainly survive in some regions from which mountains at present exclude them.

As examples of the first possibility let it be

supposed that perch could cross the Himalayas into Pakistan and northern India. It is true that below the slopes of the Himalayas large, mature rivers are encountered, which might seem suitable. But air temperatures in midsummer are exceedingly high (of the order of 38°C.) so that even with the low relative humidity prevailing in this region it seems very doubtful that these streams would be thermally tolerable. In these latitudes, even in the cooler part of the year it is about as hot as in the North American midsummer, and humidity is high. Similar conditions obtain in southern Persia and China in summer, and higher humidity prevails than in northern India.

Manchuria furnishes an instance of the second possibility. Here air temperatures are warm but not unduly high and large mature rivers and lakes are present. Perch could probably live in Manchuria if they should gain access in sufficient numbers to become established.

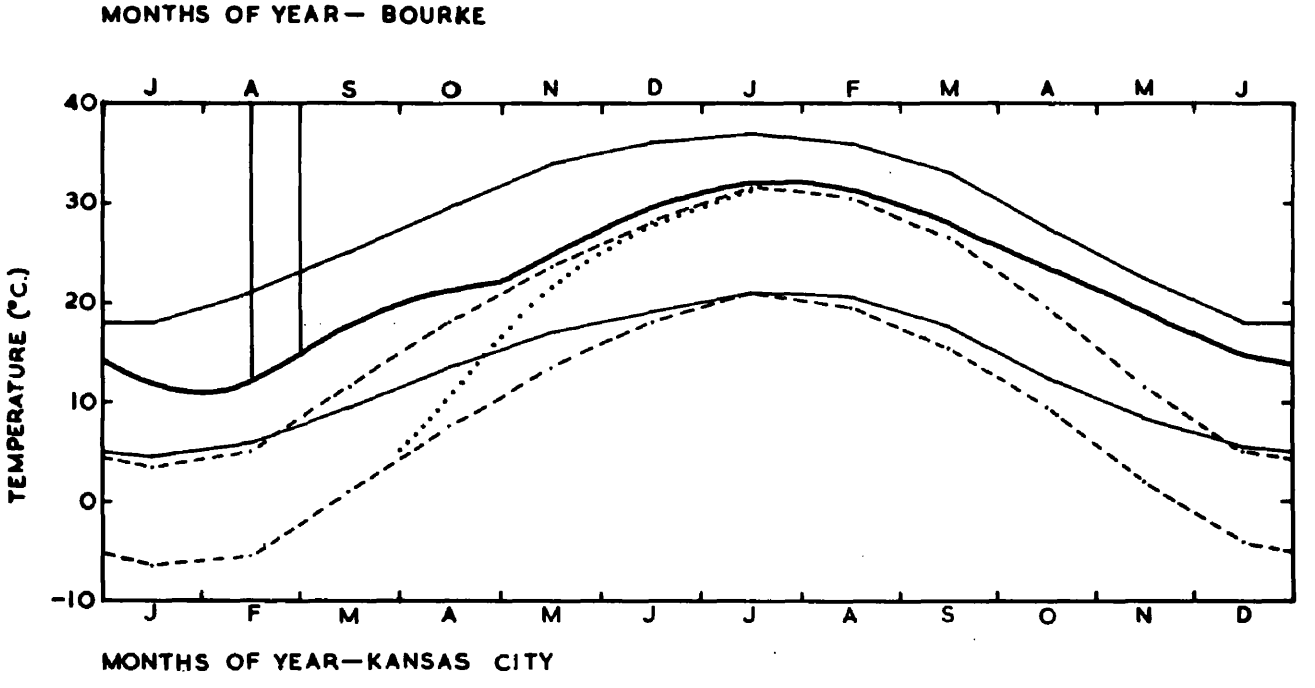
.....

This study has not included an investigation of the effects of higher temperatures on perch during the breeding season, i.e. on their developing gonads and fertilized ova. In the first place perch spawn in spring, thus missing the hottest part of the year during this phase of their life

cycle, and ensuring that the bulk of any 0 group fish would be well beyond the larval, and immediately post-larval, stages of their lives before encountering the worst heat of summer. However, it may be of interest to glance at Figure 14. Here are shown the annual cycles of air temperature, and water temperature for the Darling River, at Bourke, New South Wales. The curve for the Darling at Bourke was constructed from data kindly supplied by J.S. Lake, 1960; he has given his assurance, founded on considerable experience of the region, that temperatures in the river at Bourke are very similar to those at Wilcannia (Figure 9), which, it will be recalled, is the northernmost limit for perch in the Darling (Lake, 1959). These curves are contrasted with the mean midsummer water temperature in the Fairport region of the Mississippi (from Galtsoff, 1923-4), together with air temperature curves from Kansas City which is not far away. The main point to note is the much smaller amplitude between summer and winter air temperatures and water temperatures in the Australian environment. This has the effect that water temperatures in this region of the Darling (the record is very similar, though not quite so complete for Wilcannia) remain above 21°C. from mid-October until early May. Indeed, in the whole world range

Figure 14. Water temperatures in the Darling River, and air temperatures, at Bourke (New South Wales), in the Fairport region of the Mississippi River and at Kansas City. Vertical lines striking the temperature curve for the Darling River indicate the spawning period in it for P. fluviatilis, and the corresponding water temperature.

——— mean monthly maximum & minimum air temperatures at Bourke; - - - - - - - mean monthly maximum & minimum air temperatures at Kansas City; ——— curve of water temperature in Darling River at Bourke; ..... curve of water temperature in Mississipi River at Fairport.


MONTHS OF YEAR— BOURKE
J A S O N D J F M A M J
40
30
20
10
0
-10
TEMPERATURE (°C.)
J F M A M J J A S O N D
MONTHS OF YEAR—KANSAS CITY

of perch the New South Wales environment probably provides the most equable yearly thermal conditions, and the warmest spring. The North American spring, even on the southern fringe of perch occurrence, will present considerably cooler water conditions, to judge from the numerous air temperature data. In England and Europe the spawning period for P. fluviatilis extends from mid-March to June - but usually April and May - (Schindler, 1957), varying according to prevailing physical conditions in the water (especially temperature, light (?), etc.). In Lake Windermere most perch spawn in May (Le Cren,1951) when the temperature is about 11°C. (Jenkin, 1942). As Figure 14 shows, temperatures in the upper Darling River may be several degrees warmer than this during the spawning period there of P. fluviatilis. P. flavescens spawns in North America over much the same period as P. fluviatilis in Europe (e.g. Pearse & Achtenberg, 1917-18). It may probably be concluded that if high temperature is not unduly affecting perch during their life cycle in New South Wales it is not likely to be doing so elsewhere.

Nevertheless, it is possible at least for some fish species that early thermal history may profoundly affect their temperature tolerance in later life, irrespective of

future acclimatizations. Thus Gibson (1954) found that Lebistes was affected by its early temperature background to a considerable degree, at least in relation to laboratory tests of temperature tolerance. She suggested that apparent "geographic" differences in lethal temperature which Hart (1952) reported for several species of fish, may have been due to the early thermal background of the various populations he examined. If this view is tenable it could be argued that perch from places where the young fish are exposed to warmer conditions might have different thermal tolerance than those from cooler climates. However, if there is such an effect it seems unlikely to be large enough to be of much biological significance, and is certainly too small (if present) to be appreciated in the present study.

.....

As pointed out in the Introduction, certain workers, especially those concerned mainly with Salmonidae, have suggested that fish rarely encounter lethally high temperatures, or anything approaching them, in nature, even though there may be a recognisable relation between upper lethal temperatures and habitat type for various species.

In recent years several workers have investigated the question of temperature selection, or preference, among

fish, paying particular attention to the effect of acclimatization temperature (Fry, 1951; Sullivan & Fisher, 1953; Pitt, Garside & Hepburn, 1956; Garside & Tait, 1958). It is difficult to estimate the significance of such studies, since they depend mainly on laboratory investigations with a rather stereotyped approach, and corroborative field data are usually lacking. However, work on the speckled trout Salvelinus fontinalis, summarized by Fry (1951), is more complete than for most other species, and it appears likely that this species does actively avoid high temperatures in the field. Thus Creaser (1930) showed that speckled trout were absent from streams where temperature exceeded 19°C. in summer, even though such streams were in other ways (including chemical properties) quite similar to those where temperatures were below 19°C. and in which speckled trout flourished. Fry (1951) has described how speckled trout acclimatized to less than 14°C. seek warmer water, and above 19°C. seek cooler water. In the range 14 to 19°C. they tend to remain in the temperature to which they have been acclimatized. The upper preference temperature of 19°C. is well below the highest temperature tolerable for a prolonged period (25.3°C.). The work of Graham (1949) indicates that between 16 and 20°C. is the optimum temperature for the

speckled trout, because it is able to move and respire most effectively within this range. That is to say that both the greatest speed which speckled trout can maintain indefinitely and the greatest difference between their oxygen uptakes during minimal and maximal activity were found in this temperature range.

Information on these subjects of temperature preference, respiration and activity seem to be lacking for P. fluviatilis and P. flavescens, but as already indicated they can occur in water where temperatures can, at least temporarily, exceed 30°C. And in the unnatural environment of the aquarium tank they can live at 30°C. without superficially unfavourable symptoms, save perhaps, weight loss and excessively high activity. It seems clear then that perch can live within a degree or so of their thermal limit for prolonged periods, and they certainly do not indulge in the sensitive avoiding reactions to dangerously high temperatures which seem to characterise at least some of the Salmonidae. Yet just above this critical level of temperature perch die in the laboratory if exposed for any considerable length of time, nor are they found in waters significantly warmer than this in nature.

Now it could be argued that perch must exercise

temperature selection so as to remain just below their lethal level in nature. But it seems simpler to suggest that their spread towards the equator is prevented by the direct effects of upper lethal temperatures in those parts of the world where other limiting factors, such as stream gradient, or isolation of one river system from others, are not the principal ones. It is hoped to reinforce this viewpoint in the sections which follow, and to demonstrate that the ability of perch to live so close to their lethal temperature is only obtained at the expense of a considerable call upon their biological resources.

## IV. EFFECTS OF EXPOSURE OF PERCH TO HIGHER TEMPERATURES.

### (a) Results of Histological Examinations.

Reference should again be made to Figure 1 and Table 3 to appreciate the nature of the various exposures or acclimatizations; results described here will be in the order of the Experiments given in Table 3. Effects of prolonged high temperatures, of moderately high temperatures and of lethal and near lethal temperatures will be recounted under the headings of the various tissues and organs examined, in the following order: Head kidney, interrenal tissue, renal tubules, spleen, liver, thyroid, axial muscles.

(1) Head kidney.

In most teleosts the head kidney is basically derived from pronephros, largely through replacement by lymphoid tissue. Baecker's (1928) account of the head kidney of P. fluviatilis is very good, especially as it was based on only two specimens, being part of a wide comparative survey of head kidneys of many teleost species. But his descriptions do not make it easy to visualise the shape of the head kidney, and his account fails to consider the morphology of the kidney proper, or its blood supply. Part VI. of this thesis describes the blood vascular system of these organs and certain other points of their morphology.

For present purposes it is sufficient that the head

kidney consists of two lobes composed largely of lymphoid tissue, conjoined medially by a narrow bridge of the same tissue, each lobe situated at the anterior end of one of the kidneys. The cardinal veins, each of which traverses one lobe of the head kidney, have, arranged around their lumina within the head kidney, the interrenal tissue which may vary from one to many cell layers in thickness. But even at any one location along the course of the veins these layers tend to vary in thickness, and are usually arranged discontinuously around the lumina of the veins.

In low temperature control fish (i.e. perch acclimatized to low temperatures) the main substance of the head kidney consisted of dense masses of lymphocytes arranged in a close-knit, compact reticulum, interpenetrated by fairly obvious blood capillaries and numerous narrow blood channels (Plate 1, Figure 1). In the reticulum are found both large and small lymphocytes, with the latter predominating. Yoffey (1929) gave an excellent account of the various cell types, which may arise from an origin as small lymphocytes in the spleens of teleosts and elasmobranchs, and it has been inferred from appearances that much the same applies to the lymphoid tissue of the head kidney.

The effects of rapid and severe temperature stress, as when (Experiments 1 & 5, Table 3) temperature was elevated from an acclimatization level of about 7.5°C. to a point just

Plate 1. Appearance of perch head kidney.

Figure 1. Dense lymphoid mass of head kidney of perch acclimatized to low temperature (7.5°C.). Note narrow blood channels.

Figure 2. Head kidney of perch just before disablement during lethal test; acclimatization temperature 7.5°C. Channels have here become much wider and contain many red cells and lymphocytes.

Figure 3. Head kidney of perch after death in lethal test; had been held at 17.5°C. for 2 days before test. Widespread lymphoid depletion with broad channels.

All X 600.

Plate 1.

Fig. 1

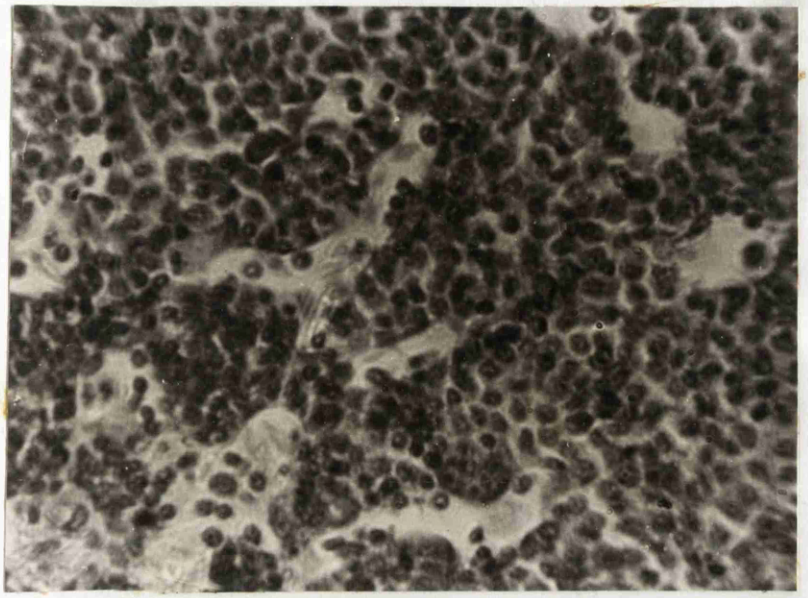


Fig.2

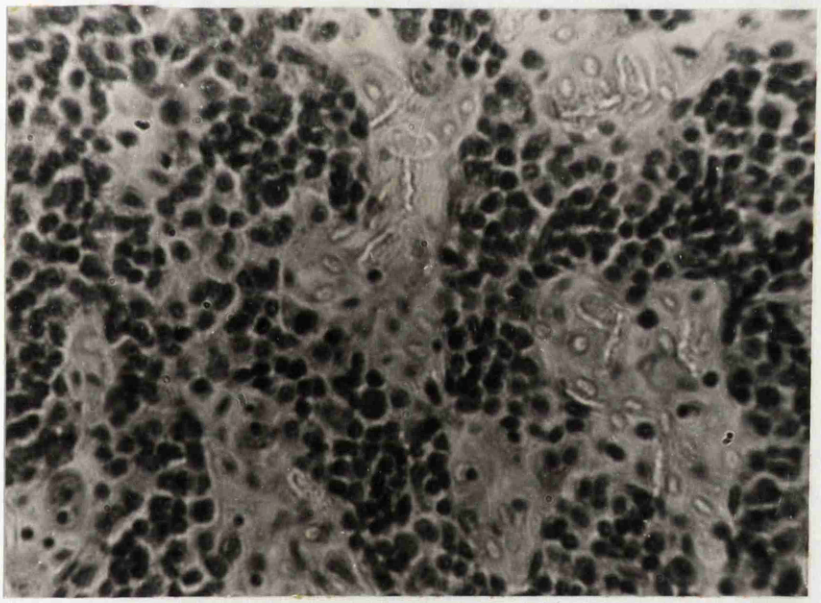
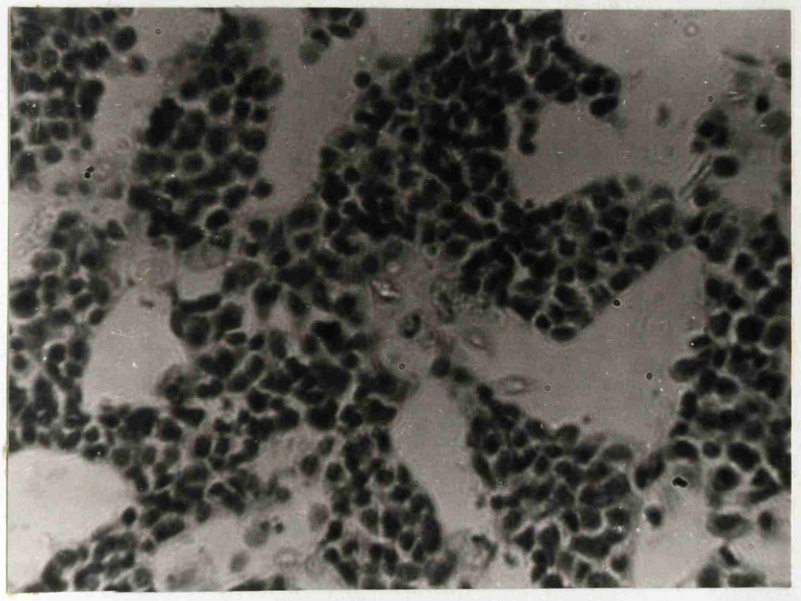


Fig. 3



before disablement, at 4°C. per hour, produced the results pictured in Plate 1, Figure 2. Marked lymphocyte losses have occurred, so that the channels have increased greatly in extent and are much wider. The lymphoid tissue remaining is still firmly packed. In another perch removed before disablement and in two fixed immediately after death the appearance of the head kidney was very much the same as this. In interpreting this type of lymphoid depletion it has been inferred that an increased blood flow accompanying rising temperature and activity has simply swept away the lymphocytes lining the blood channels.

In Experiment 7 the condition of the head kidney in fish exposed for 2 days to 17.5°C. following rapid elevation to that temperature, resembled that for the stressed fish of Experiment 5, except that the blood channels were less wide but more numerous. But after death, following a lethal test produced by heating from 17.5°C., advanced lymphoid depletion of the head kidney was found, about half its substance being lost to the circulation. Most of the channels had become wide and branched freely, and many of the packed lymphocytes seemed to have become isolated into separate masses (Plate 1, Figure 3).

The loss of lymphocytes in head kidneys of fish held at 17.5°C. for 7 or 18 days (Experiments 3 & 6, respectively) was similar, though apparently less severe than in Experiment 7, but in lethal tests, based on fish from both these

experimental batches, heavy losses of cells were apparent before disablement or after death.

Among fish exposed to 20°C. for 6 or 30 days (Experiments 4 & 10) only slight to moderate lymphocyte losses were seen, so that it seems fairly likely that some measure of lymphoid recovery is possible in fish exposed to moderately high temperatures for sufficiently long periods, though as usual during lethal tests, before disablement and after death, lymphocyte depletions were severe.

Extensive and irregular channelling of the lymphoid tissue was seen among the fish from Experiments 11 & 12, which represented those acclimatized to very high temperatures (28 and 30°C.). Here the edges of the channels were no longer at all clear cut, as typified in Plate 1, Figures 2 & 3, but uneven; the overall appearance was that of a broken maze.

The conception of the head kidney which emerged from this examination under varying thermal conditions was that of a dense lymphoid mass, capable of losing many of its cells rapidly via its blood channels - which themselves progressively widen - when temperature is elevated. But in addition, the lympho-reticular structure of the head kidney seems to be permanently less closely-knit in perch acclimatized to very high temperatures. The condition of the tissue may be

largely determined by the higher rate of blood flow through the head kidney at higher temperatures. Certainly whenever blood flow could be presumed to be very high the head kidney rapidly lost many of its lymphocytes and the blood channels could often be seen to be packed with huge numbers of erythrocytes and some lymphocytes.

(ii) Interrenal tissue.

Critical appraisals by such workers as Aboim (1946) and Chester Jones (1957) appear to have established with certainty the homology of the interrenal tissue of fish with the mammalian adrenal cortex. This homology was accepted by Rasquin (1951) and Rasquin & Rosenbloom (1954), in work which included observations on the interrenal tissue of Astyanax, and is also accepted in the present study. Chester Jones (1957) described the *"basic unit of the vertebrate (adrenal) cortex"* as *"essentially, a cord of cells...."*. He also demonstrated some of the ways in which growth, including the more or less complex looping of this basic cord of cells, may occur within the various vertebrate groups. Perch interrenal tissue often shows a cord-like arrangement of its cells, and the cells also tend to become arranged in the form of hollow cylinders, which are to be seen whether the fish have been exposed to higher temperatures or not, though the tendency is greater at the higher temperatures. This point will be returned to later.

In perch living at low temperatures (6-8°C.) interrenal cells were characteristically rectangular, cuboidal, subcuboidal or polyhedral in shape, with a very distinct, markedly spherical nucleus. As revealed by the adopted methods of fixation and staining the rather considerable cytoplasmic mass of these cells had a fairly granular appearance. These findings accord well with those of Baecker (1928) who also described the perch interrenal tissue. Baecker rather aptly referred to the prominent, globular nucleus as *"bubble-like"*. The cell cytoplasm was acidophilic and the nucleus basophilic with haemalum and eosin, which again agrees with Baecker's finding (he used the same stains, among others). Figure 1, in each of plates 2 to 6 show characteristic examples of interrenal tissue of the perch from low temperature control (unstressed) fish, since, naturally, its appearance has a certain variability from one fish to another.

Now in Experiments 1 & 5, the appearance of interrenal tissue in perch after severe temperature stress, but before disablement, differed markedly from that in the low temperature controls. The cuboidal shape of many of the cells had become emphasised, with the angles of the cells more clear-cut and the margins very clear, as if some shrinkage of cytoplasmic contents had occurred. Many cells seemed more

Plate 2. Interrenal tissue of perch from Expt. 5.

Figure 1. In the right top corner may be seen lymphocytes of the head kidney, to the left erythrocytes in the lumen of the cardinal vein. Between them are the interrenal cells. This is a section from a fish acclimatized at 7.5 deg. Cent.

Figure 2. Fish removed just before disablement in a lethal test. Cylinder formation apparent in interrenal tissue, also some distortion and atrophy of cells. Some lymphocyte depletion seen at left.

Figure 3. Interrenal tissue of perch immediately after heat death. Erythrocytes fill spaces among cells.

All X 600.

Plate 2.

Fig. 1

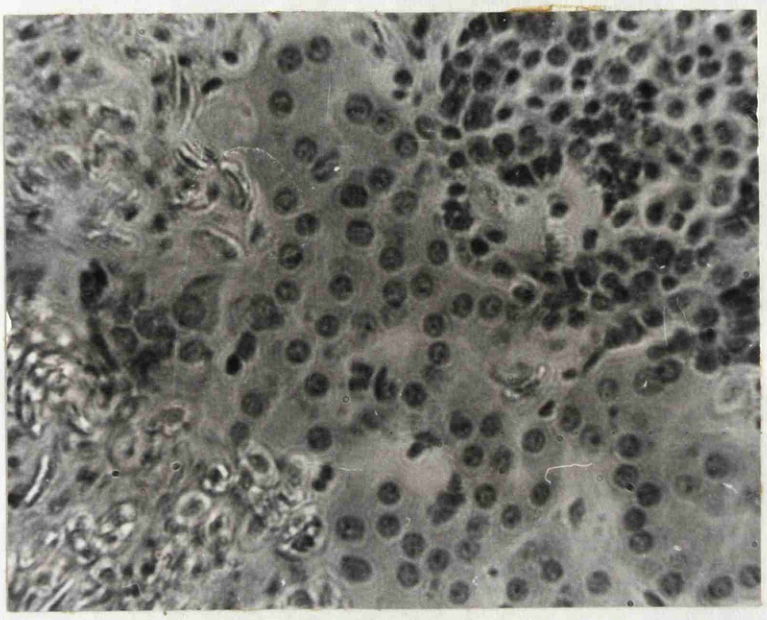


Fig. 2

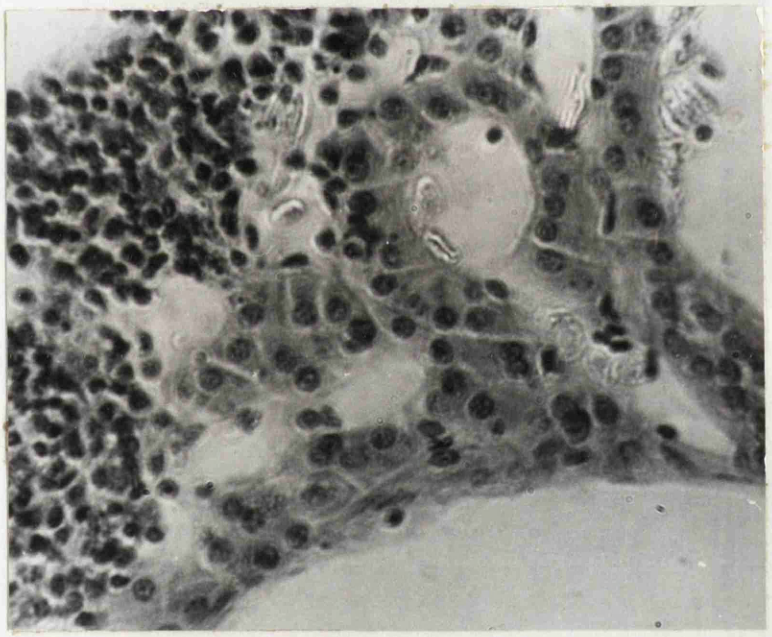
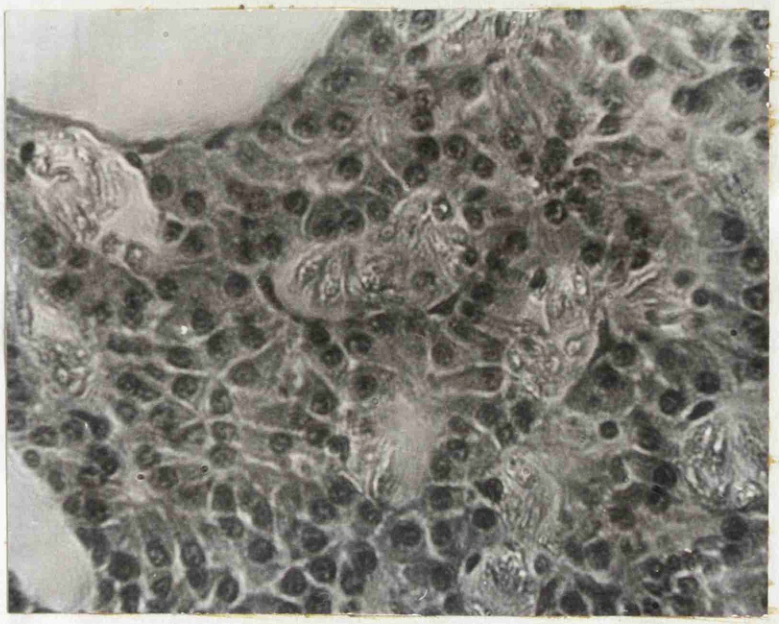


Fig. 3



elongated, though this proved to be merely an impression derived from their having shrunk more in one dimension than in others. This cellular atrophy was accompanied by a change in position of many of the centrally located nuclei, which now appeared, at least in section, to occupy one end of the cell. The cylinders of interrenal cells, often one cell thick, were of common occurrence, and these, together with the other spaces in the interrenal tissue, frequently had blood cells in them (Plate 2, Figure 2). In these two experiments, fish after death had interrenal cells which looked even more angular and atrophied. Cell margins had become still clearer and more conspicuous, and many of the cells were now quite tear-shaped as seen in section, broadest at the nucleus, which in many instances occupied one end, and tapering to a point at the other end. Others were somewhat fusiform or approximately spindle shaped (Plate 2, Figure 3).

After 2 days exposure to 17.5°C. (Experiment 7), perch interrenal tissue resembled that of fish in the lethal test of Experiment 5, only that now the cells had definitely become longer and so more rectangular, thus appearing in many instances to have hypertrophied initially and subsequently atrophied (Plate 3, Figure 2).

Plate 3. Interrenal tissue of perch from Expt.7.

Figure 1. Low temperature control: interrenal cells of fish acclimatized at 7.5 deg. Cent. To the right a compact mass of head kidney lymphocytes.

Figure 2. Interrenal of perch exposed to 17.5 deg. Cent. for two days. On the right depleted lymphoid tissue, on the left erythrocytes in cardinal vein. Between them layer of interrenal tissue; appearance suggestive of initial hypertrophy followed by atrophy.

Figure 3. Interrenal of perch immediately after lethal temperature exposure. Note great cellular atrophy, marked cord & cylinder arrangement, massive lymphocyte depletion on right, with clearly-defined, broad channels through head kidney.

All X 600.

Plate 3.

Fig. 1

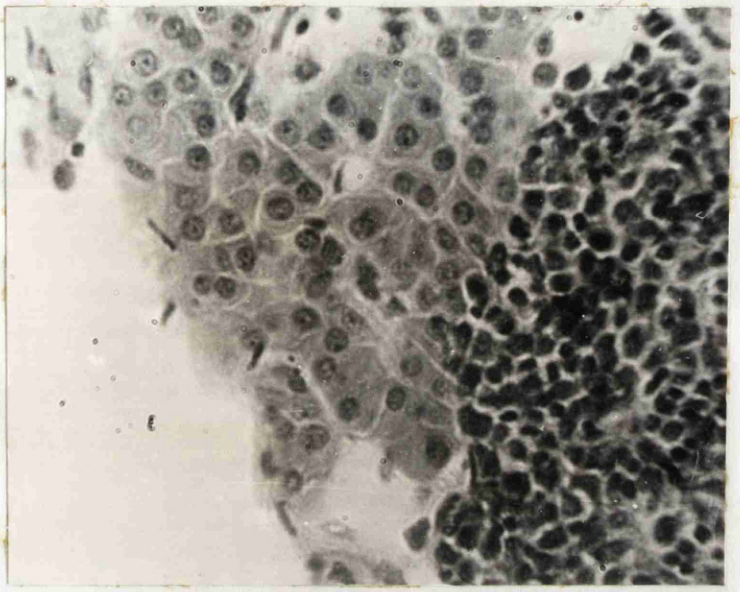


Fig. 2

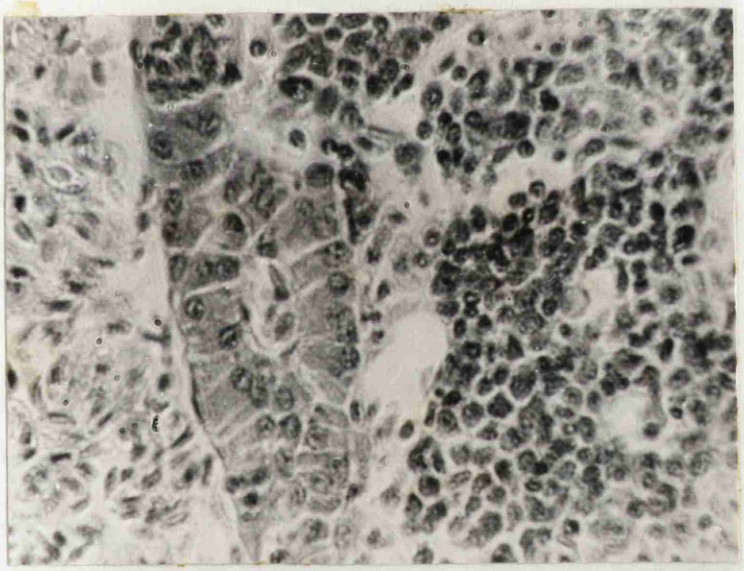
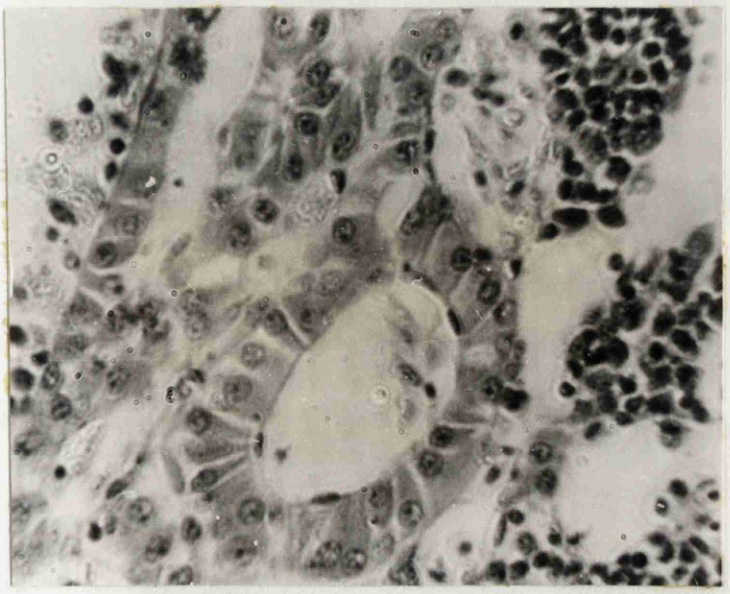


Fig. 3



After death, induced by elevation of temperature in a lethal test, cells were in many cases reduced to tear-shapes, spindle-like and fusiform shapes, pointed and wasted rectangular and polyhedral figures. As a result of this striking atrophy the cell margins were exceptionally clear, and considerable numbers of cylinders were prominent (Plate 3, Figure 3).

The appearance of interrenal tissue from fish in Experiments 3 and 6 (exposure to 17.5°C. for 7 and 18 days) was similar to that after 2 days at 17.5°C., but with certain differences: In Experiment 3 the cells had definitely hypertrophied (Plate 4, Figure 2), while in Experiment 6, if they had hypertrophied initially they had subsequently shrunk again, so that they were even more distorted than those in Experiment 7. The added effects of a lethal test produced the degree of atrophy of the cells noted in other Experiments (Plate 4, Figure 3).

In Experiment 4 the interrenal tissue of fish held 6 days at 20°C. looked stressed, though, as usual, the picture just before disablement and after death was more severe. But for fish held 30 days at 20°C. (Experiment 10) and for those of this group subjected to a lethal test, appearances of the interrenal tissue

Plate 4. Interrenal tissue of perch from Expt. 3

Figure 1. Interrenal tissue of low temperature control fish acclimatized at 7.5 deg. Cent.

Figure 2. Definite hypertrophy of interrenal cells following 7 days at 17.5 deg. Cent. Note usual lymphocyte depletion and renal tubule at lower right.

Figure 3. Though some atrophy of interrenal cells has occurred just before thermal disablement, it has not yet become extreme.

All X 600

Plate 4.

Fig. 1

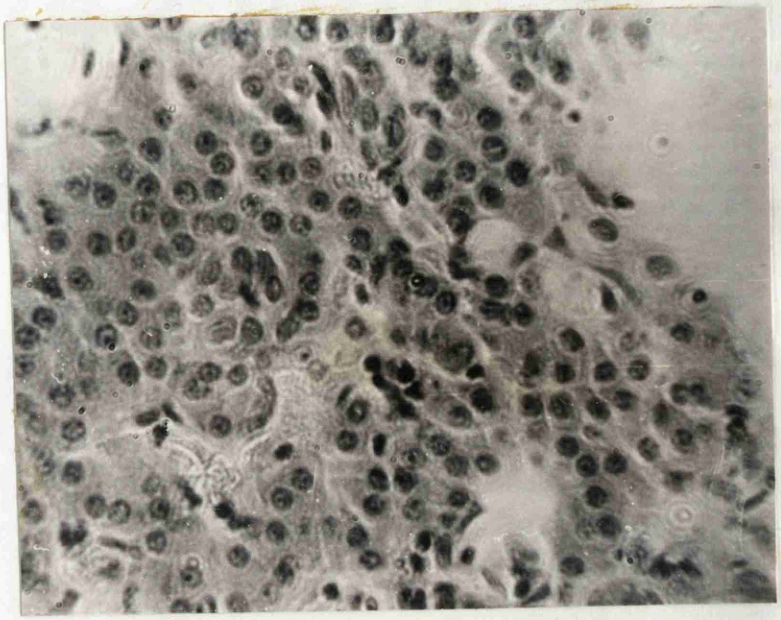


Fig. 2

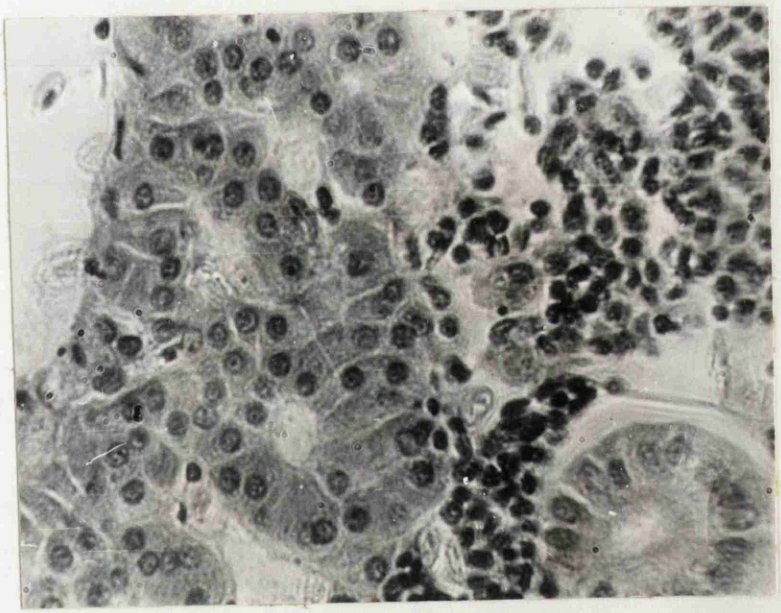
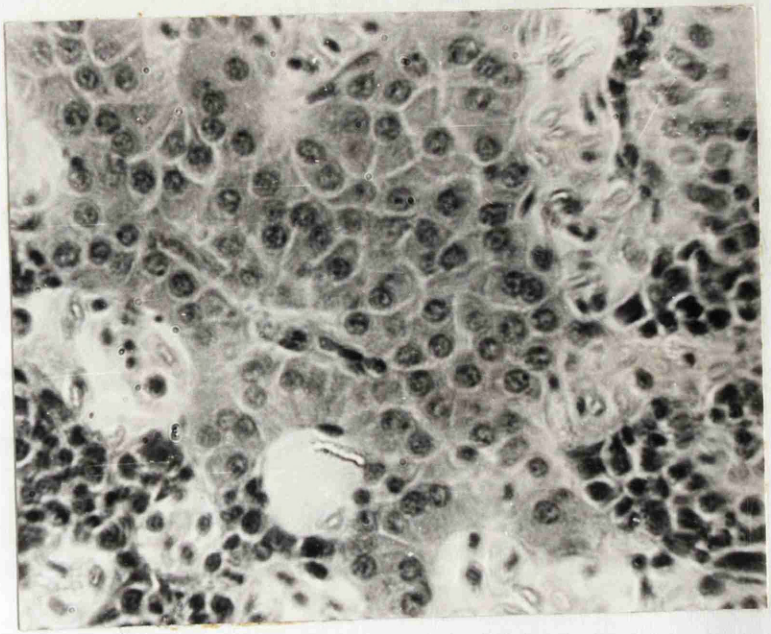


Fig. 3



signified heavy stress (Plate 5).

The most significant differences between the appearance of interrenal tissue of fish exposed to high temperatures and those held in the low range was seen in sections from fish in Experiments 11 & 12 (16 days at 28°C., and 7 days at 30°C., respectively; see also Figure 1). Interrenal tissue of fish from both these experiments had greatly atrophied cells, some appearing as merely a thin covering of cytoplasm investing a still-spherical, and apparently undamaged, nucleus (Plate 6). In one of two fish fixed after death, following a lethal test on the batch of fish in Experiment 11, the cytoplasmic investment of many of the cells was reduced to such a slight vestige that much of the interrenal tissue looked almost like collections of nuclei alone.

Though by no means certain in the earlier experiments, in Experiments 11 and 12 there was definite evidence of interrenal hyperplasia. That this was not immediately apparent on examination was due to the very considerable cellular atrophy which had occurred, which tended to mask the hyperplastic condition.

(iii) Renal tubules

Sections through the head kidney permitting

Plate 5. Interrenals of perch from Expt. 10.

Figure 1. Interrenal tissue from low temperature control fish, acclimatized at 7.5°C.

Figure 2. Fish held 30 days at 20°C.; interrenal atrophy plus moderate lymphoid depletion.

Figure 3. From fish acclimatized to 20°C. then subjected to lethal test; removed just before disablement. Note pronounced interrenal atrophy; cytoplasm of many of the cells so reduced as to appear as mere rim surrounding apparently undamaged nucleus.

Figure 4. Much as for Figure 3, but from fish immediately after thermal death.

All X 600.

Plate 5.

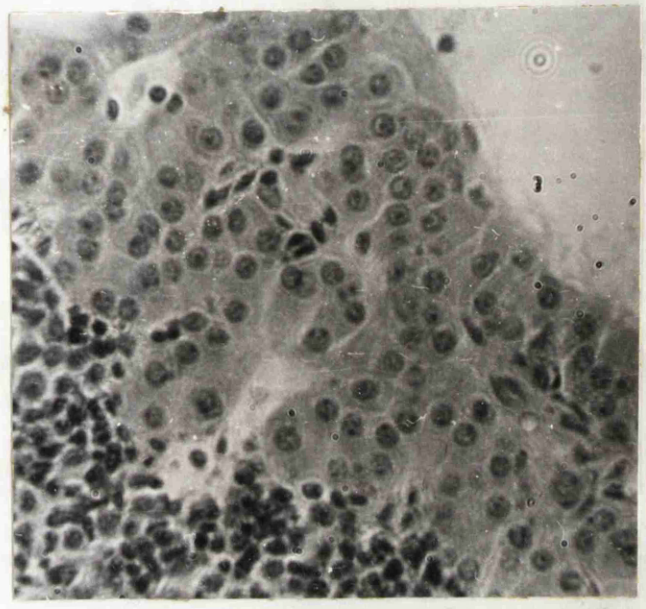


Fig. 1

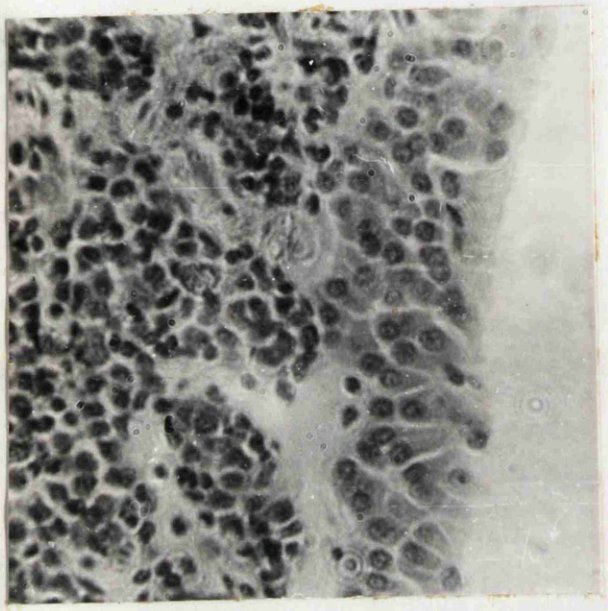


Fig. 2

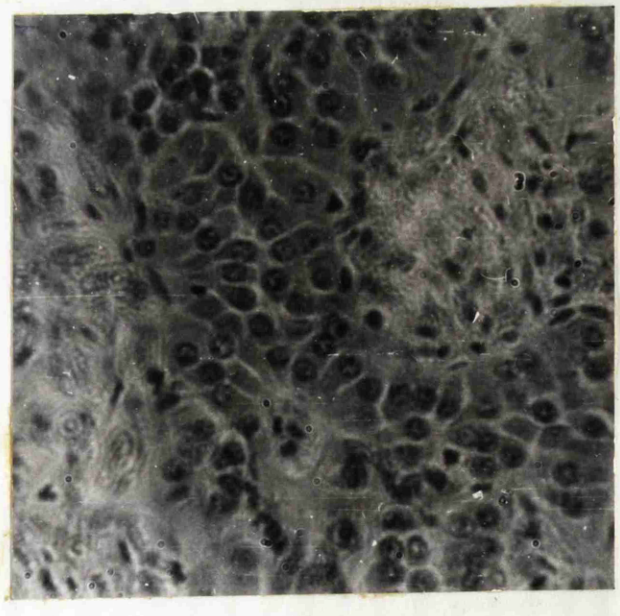


Fig. 3

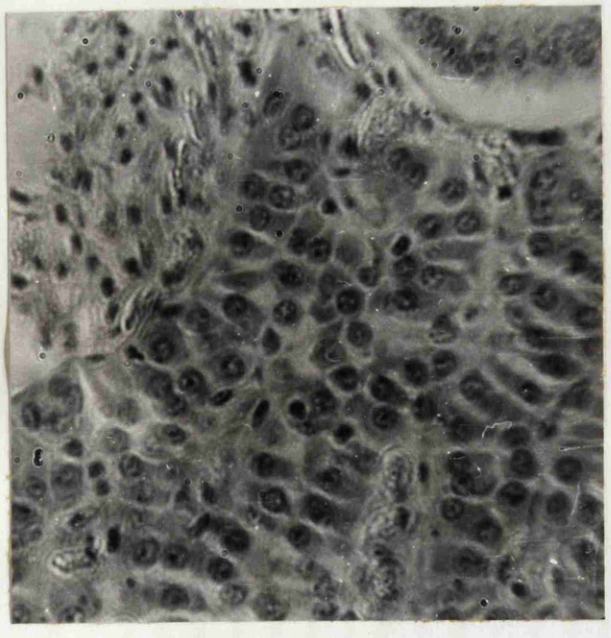


Fig. 4

Plate 6. Interrenals of perch from Expt. 12.

Figure 1. Interrenal tissue from low temperature control fish acclimatized at 7 deg. Cent.

Figure 2. Fish held 7 days at 30 deg. Cent. Interrenal tissue, to the top and right, is greatly atrophied and interpenetrated by blood spaces containing erythrocytes. Lymphoid tissue - bottom & left - much depleted.

Figure 3. Much as for Fig. 2, but for fish (acclimatized to 30 deg. Cent.) just after death.

All X 600.

Plate 6.

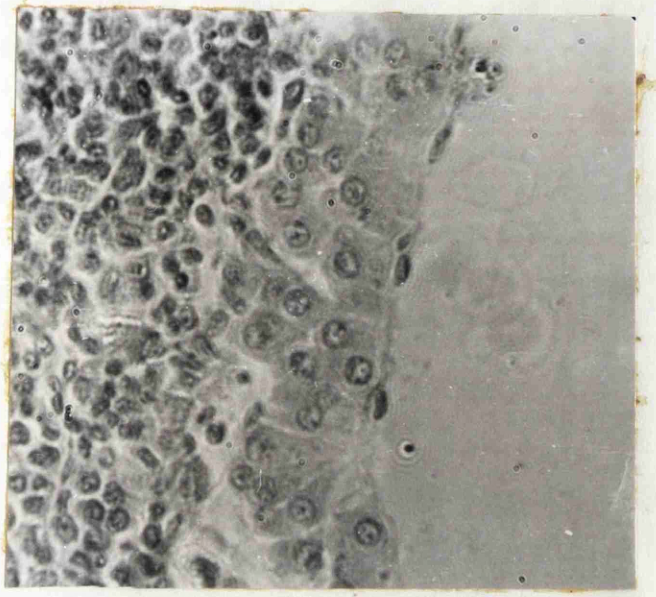


Fig. 1

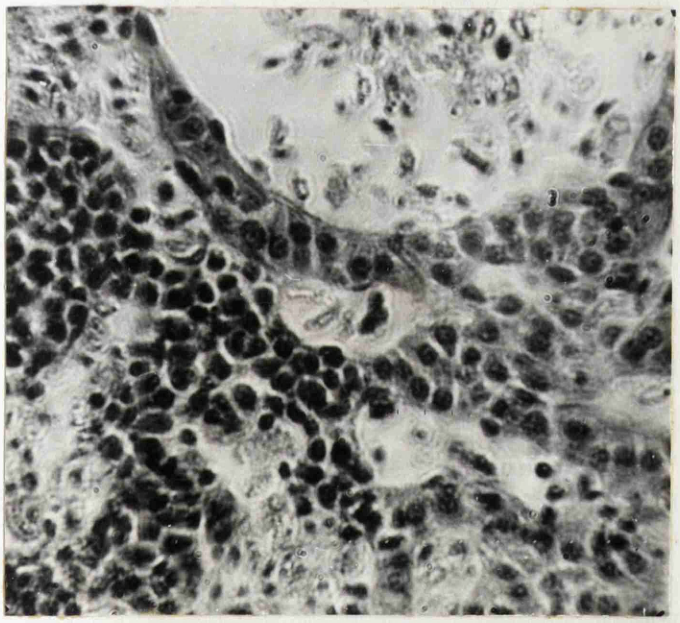


Fig. 2

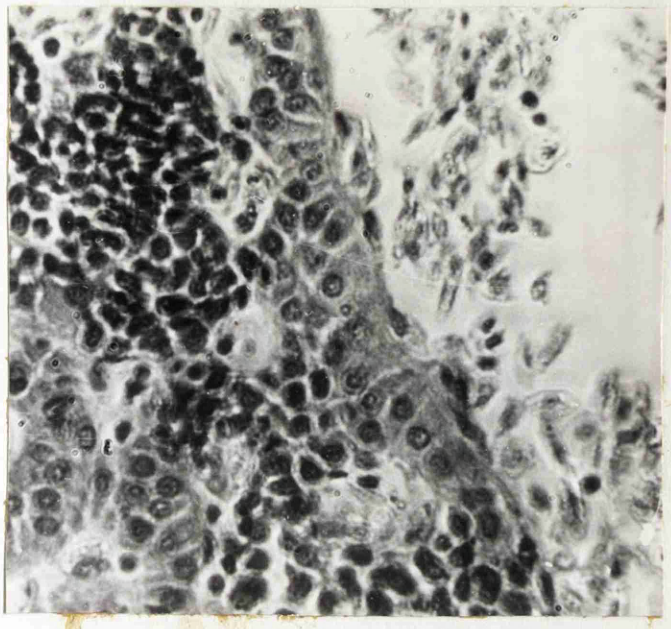


Fig. 3

examination of lymphoid and interrenal tissue also involved regions containing glomeruli and renal tubules; see Section VI. These were routinely examined whenever possible.

The characteristic appearance of the glomeruli and tubules in histological section are well enough known and require no detailed description, but it may be noted that the wedge-shaped, cuboidal, or columnar cells composing the latter had acidophilic cytoplasm and basophilic, spherical nuclei. There was no evidence from any of the experiments performed either by prolonged, moderate or short exposure to various levels of temperature, or as a result of any of the lethal tests, of any serious deterioration of renal elements to the extent that Rasquin & Rosenbloom (1954) detected in Astyanax mexicanus maintained under prolonged conditions of total darkness. However, in fish exposed to 30°C. (Experiment 12) the cells of many of the tubules had a more shrunken appearance than those from any other fish save those from two fish of Experiment 11 (16 days at 28°C.). Their appearance is pictured in Plate 7. Essentially this shrinkage or atrophy meant that many of the affected tubules appeared considerably more thin-walled than usual.

It is interesting in passing to note that Aboim (1946)

Plate 7. Renal tubules in head kidney of perch.

Figure 1. Typical appearance of renal tubules in low temperature control fish (acclimatized at 7.5°C.).

Figure 2. Renal tubules from perch held 16 days at 28°C. (Experiment 11). In contrast to Figure 1 note atrophy of both cells and their nuclei.

Both X 600.

Plate 7.

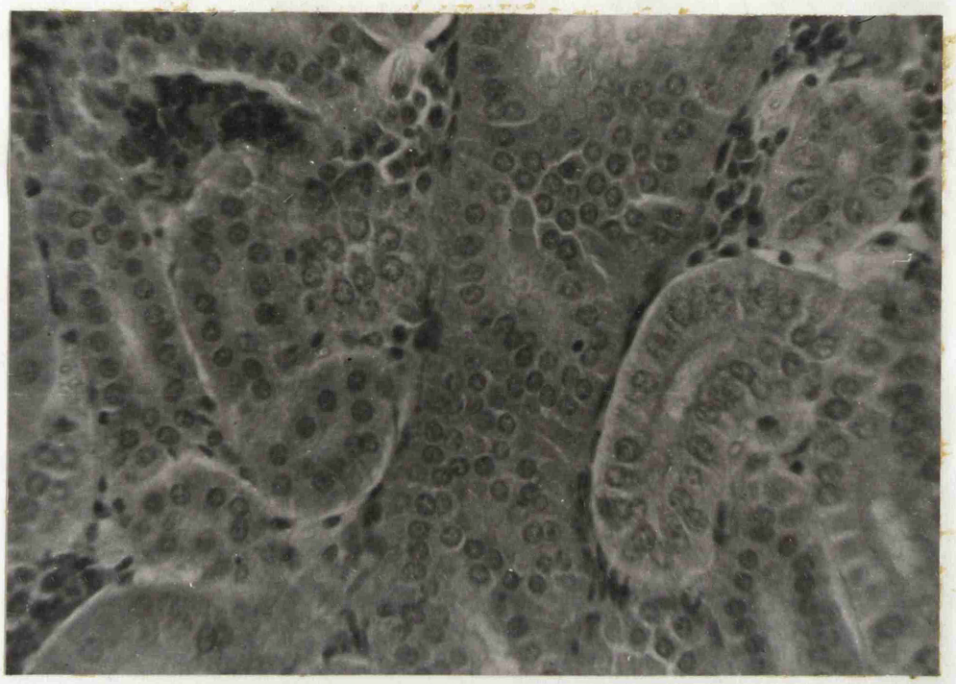


Fig. 1

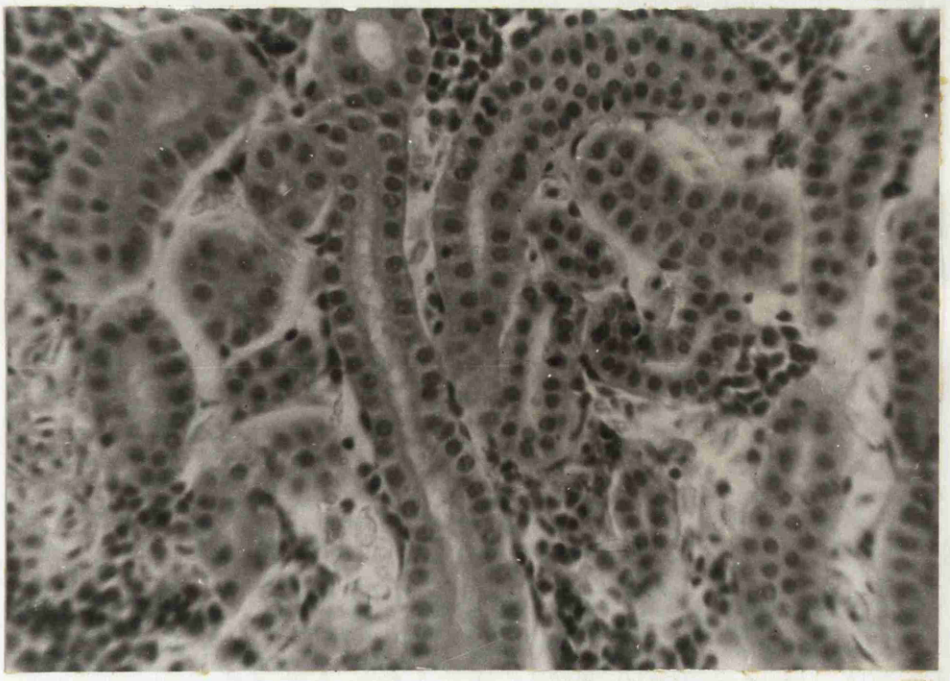


Fig. 2

points out that there is a superficial resemblance between the cells of renal tubules and those of the interrenal tissue, and that this caused some confusion in the past.

(iv) Spleens

Yoffey's (1929) excellent account of elasmobranch and teleost spleens was found useful in elucidating the structure of the spleen of P. fluviatilis, and evaluating the changes produced in experiments. While no attempt was made to study the detailed structure of the spleen for its intrinsic interest, the following general notes of observations made during the present work may be appropriate here, especially since the spleen of the perch does not seem to have been the object of much previous examination.

The perch spleen has the simple elongate shape commonly associated with this organ in the various vertebrate groups, and is almost invariably triangular in cross-section. It lies in close approximation to a loop of the anterior part of the intestine. Something of the structure of the spleen can be made out when a large part of its normal contents have disappeared through the action of the blood stream during the last

phases of a lethal temperature exposure. The capsule appears to be of simple, non-muscular structure, as Yoffey (1929) showed was commonly the case for many teleosts, and as his account indicated, the spleen has a large central vein and a main artery, which is conspicuously thick-walled, though not particularly wide. The numerous ellipsoids (capillaries) Yoffey mentioned, which open into the pulp of the reticulum containing the lymphoid tissue, were also seen. When the perch spleen loses lymphocytes this loss is clearly featured by the relatively open spaces which show in the reticulum, which are normally densely occupied by lymphoid tissue. Sometimes these losses of cells are reflected by the presence of very large numbers of lymphocytes in the lumen of the splenic vein.

In general, the acclimatization temperatures at which perch were held in this experimental series, for whatever period, appeared to have no significant effect upon the spleen (Plate 8, Figures 1-4). The spleen is normally filled with lymphoid tissue (Yoffey, 1929, gave a comprehensive account of the cell types), so that apart from some ellipsoids and the main artery and vein structural elements can hardly be distinguished. Though the presence, in a few instances, of large numbers of erythrocytes hinted at a possible function for the

Plate 8. Appearance of the normal perch spleen.

Figures 1 & 2. Spleens from perch acclimatized at about 7 deg. Cent. Large concentration of lymphocytes masks most elements of structure, though something of the reticulum may be made out. In Fig. 1 erythrocytes are seen, especially top and centre.

Figures 3 & 4. Spleens from perch acclimatized at 20 deg. Cent. (Expt. 10) and 30 deg. Cent. (Expt. 12), respectively. They are not significantly different from the low temperature controls (Figs. 1 & 2). In Fig. 4 a splenic blood vessel has been longitudinally sectioned.

All X 600.

Plate 8.

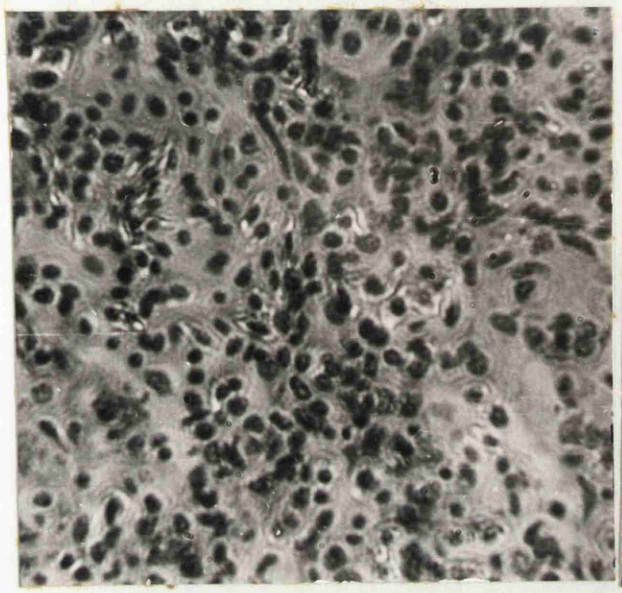


Fig. 1

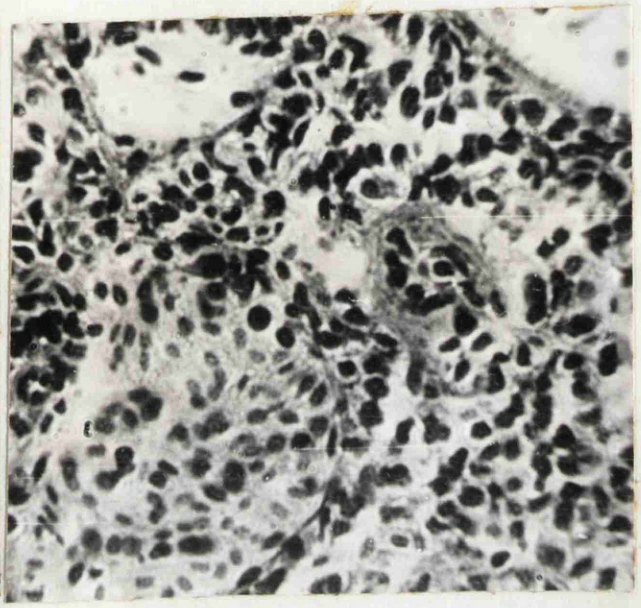


Fig. 2

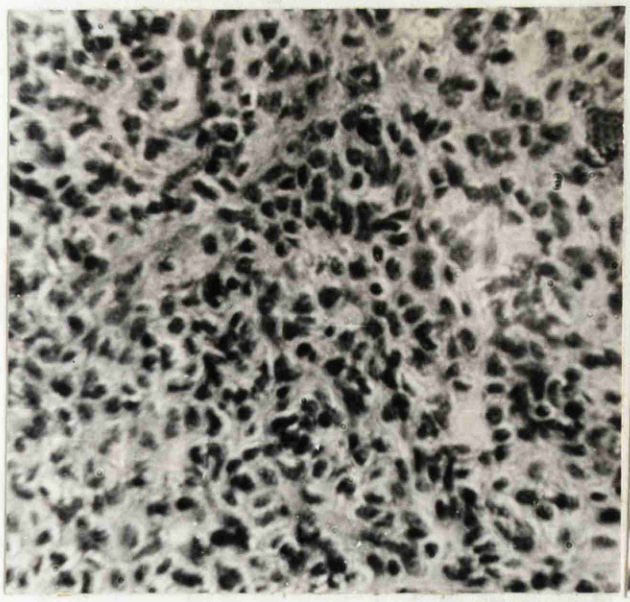


Fig. 3

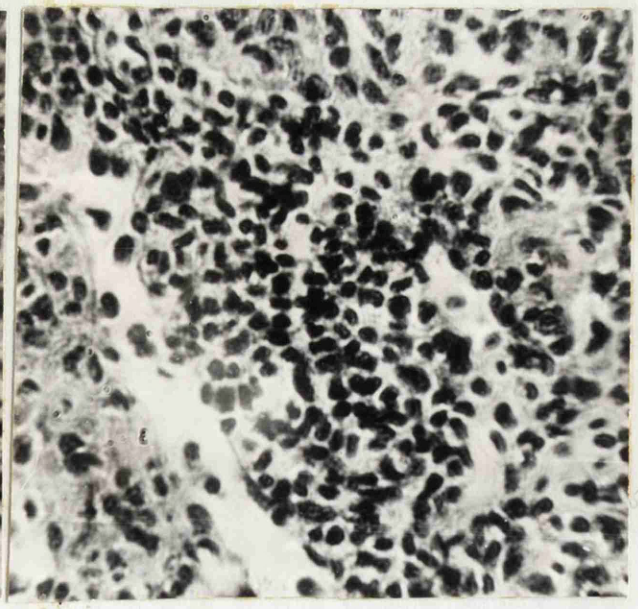


Fig. 4

spleen as a blood storage organ, the general impression obtained was that in the perch it normally functions as an haemopoietic organ.

On the other hand, in almost every case where spleens were examined from fish fixed immediately after death during lethal tests, considerable losses of cells from this organ were apparent. As indicated above, such depletions exposed the reticulum, etc. Plate 9 shows two such spleens in section. Loss of cells - very largely lymphocytes - was usually associated with a somewhat collapsed appearance of the spleen in section. As Yoffey explained, the spleen, though in fish incapable of active contraction, may undergo considerable passive change in volume to accommodate more or less lymphoid tissue or blood than usual.

As in the case of the head kidney, lymphocyte depletions of the spleen were attributed to a much increased blood flow, especially since this would be very likely to accompany the violent activity occurring as an immediate prelude to death (Section III (a) (ii)). It was perhaps surprising that many erythrocytes were not found pooled in the spleen after death, in view of its large venous supply, but perhaps the presence of elastic elements in its capsule (Yoffey, 1929), helps to expel blood from a spleen already

Plate 9. Spleens of perch immediately after thermal death.

Figure 1. From Experiment 5; perch acclimatized to 7.5°C. Spleen shows considerable lymphocyte depletion, some capillaries are visible and a good deal of the reticulum may be distinguished.

Figure 2. Much as for Figure 1, but from a perch acclimatised to 30°C. before thermal death. At top centre of picture small branch of splenic artery appears.

Both X 600.

Plate 9.

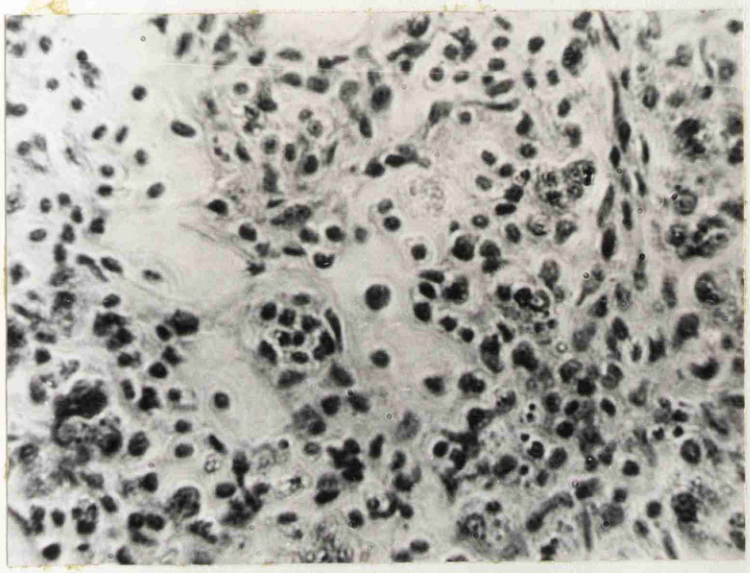


Fig. 1

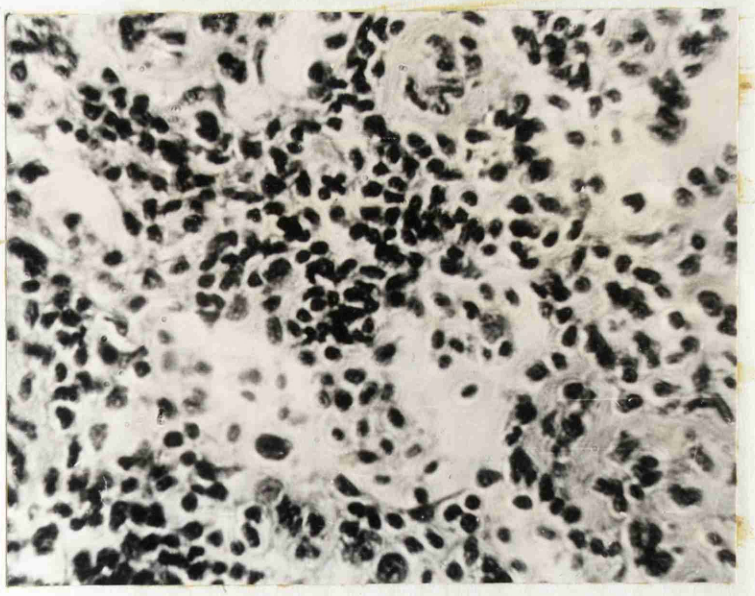


Fig. 2

impoverished of lymphoid tissue, and this would, of course, account for its collapsed appearance after death.

The pigment nodules noted in teleost spleens by Yoffey were found in a number of perch spleens. Yoffey apparently considered these nodules as products of erythrocyte disintegration, as indicated mainly by the presence in them of iron, and by their greenish-brown to brown colour. In the perch they were always amber-brown. There was no tendency for the nodules to be more prevalent in thermally stressed fish.

(v) Livers

The studies on liver morphology by Elias (1955) were found very helpful in estimating the effects of temperatures on perch livers. Elias conceives of the liver as a muralium (his term) or system of walls, forming a continuous, complexly-folded mass *"tunnelled by a labyrinth of lacunae"*. This muralium is one cell thick in the livers of the higher vertebrates, but in the lower vertebrates, including fish, is two cells in thickness; the functional and evolutionary significance of this difference has been interestingly

discussed by Elias. He also discussed the basic shape of liver cells, which vary according to their position within the muralium, the physical stresses in the liver and so forth. Apparantly the typical liver cell should be visualised in terms of figures such as octohedra, decahedra, etc., rather than as roughly cuboidal, or spherical, figures.

It was found that to appreciate the normal histological appearance of the perch liver sections from numerous fish had to be examined, for as Garven (1957) writes *"The cytoplasm (of the liver) is of very variable appearance ..... due in part to a true variation with varying functional states and in part to the changes produced by varying fixatives and subsequent treatments, e.g. the solution of contents".* With eosin and haemalum the cytoplasm of the perch liver cell stained pink or pinkish mauve, and had a granular appearance, while the predominant nuclear shade, if staining was good, was usually the kind of deeper mauve pictured by Garven (1957) for this kind of staining. Sometimes the cell walls stained clearly, at others they were faint or even indiscernible. Because of the variable appearance of the liver, Plate 10 (Figures 1, 2 & 3) pictures a selection of sections,

Plate 10. Sections of perch livers reflecting various thermal backgrounds.

Figures 1, 2, 3. Sections of normal livers from perch acclimatized at about 7 deg. Cent.

Figure 4. From perch acclimatized to 7.5 deg. Cent. (Expt. 5) subject in lethal test, but removed just before disablement.

Figure 5. Liver of perch exposed to 17.5 deg. Cent. for 2 days (Expt. 7) and then raised to its lethal temperature.

Figures 6 & 7. Livers from perch acclimatized to 20 deg. Cent. for 30 days (Expt. 10) & to 30 deg. Cent. for 7 days (Expt. 12).

All X 600.

Plate 10.

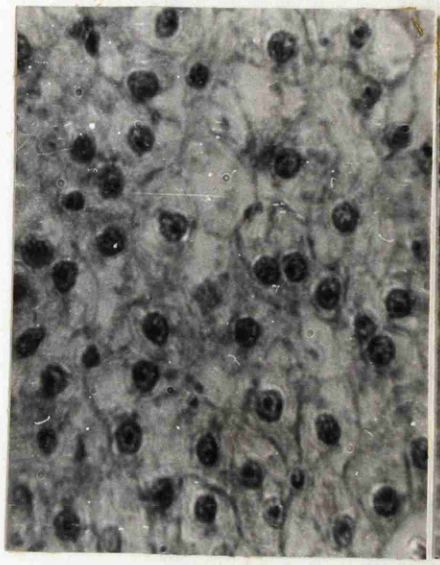


Fig. 1

Fig. 2

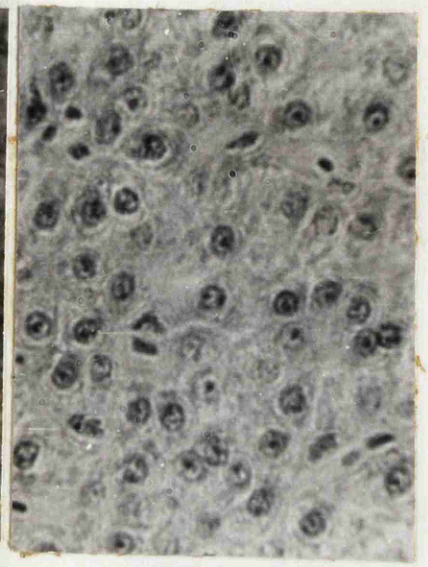


Fig. 3

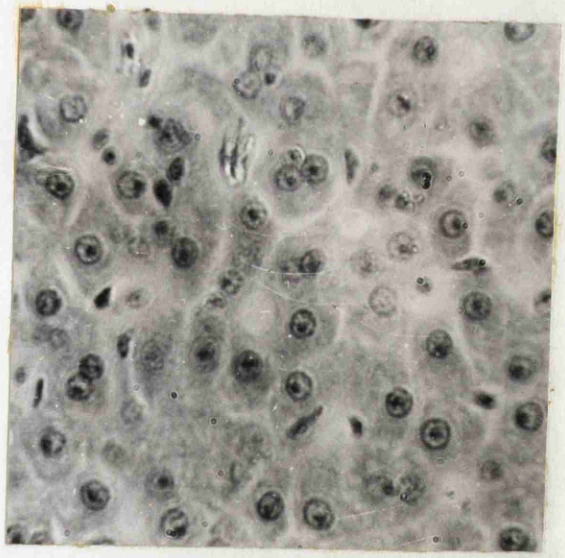


Fig. 4

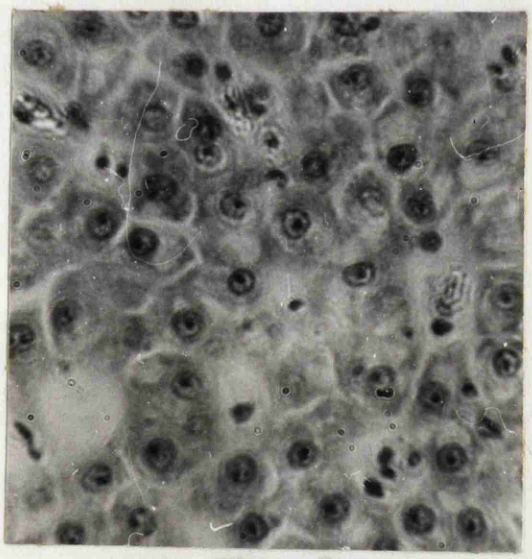


Fig. 5

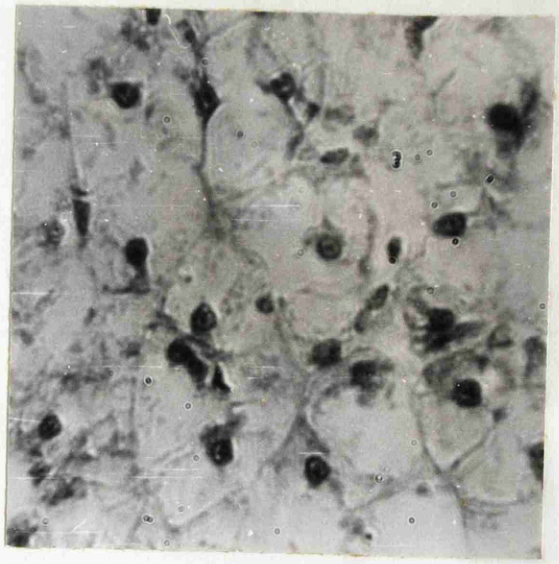


Fig. 6

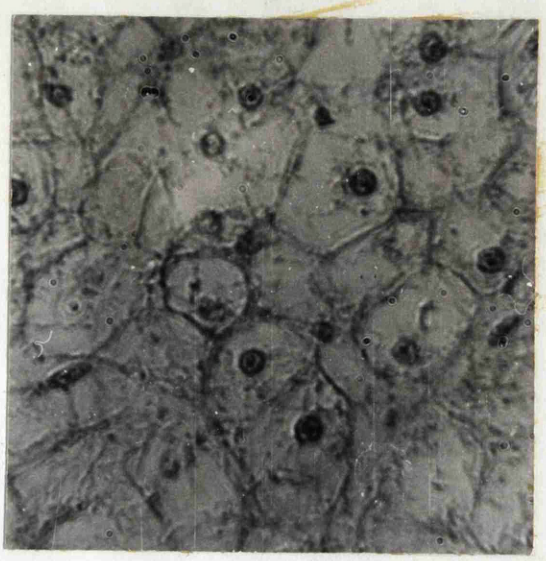


Fig. 7

indicating the range in appearance accepted as normal in this work.

Below 20°C. the temperature at which fish were held does not seem to have made any difference to the normal histology of the liver. However, in Experiments 1,3,4,5,6,7 (Table 3), among the livers of those fish fixed immediately after death at high temperatures, there was observed what has been interpreted as a partial disintegration of the organization of the liver. This was manifested in the near, or complete, separation of the cells of the liver from each other. Such a degenerative change did not affect the whole of a liver equally, but in patches the breakdown was severe, while sometimes separated cells seemed to be undergoing further, individual, disintegration. In general, such changes in the liver appear to have been a result of rapid elevation of temperature above the acclimatization level, occurring during a lethal test. Even then it seemed to have been restricted to the last few minutes of life, since such signs were usually absent from, or merely incipient in, the livers of fish fixed just before disablement (Plate 10, Figures 4 & 5). However, the changes were not an inevitable accompaniment of heat death, since not all the livers of fish fixed just after

heat death displayed it.

The livers of fish from Experiment 10 (30 days at 20°C.) varied the most remarkably from the normal in appearance as Plate 10, Figure 6 shows. In four such livers examined there was a general cellular degeneration with some necrotic patches. Many cells walls had disintegrated, many nuclei seemed to have lost their cytoplasmic investment, and nearly all the nuclei themselves were shrunken and distorted, in marked contrast to the smoothly spherical appearance of the normal nucleus of the liver cell. In many of the cells of which the wall still seemed reasonably intact, cytoplasmic contents had largely disappeared; this could signify fatty degeneration of the liver of a sort similar to that noted in Astyanax mexicanus kept in darkness as a stressing agent (Rasquin & Rosenbloom, 1954). On the basis of gross appearance of these sections it is roughly estimated that these affected livers had not more than one third as many functional cells as those from control fish, and it seems probable that even many of these were seriously impaired.

It is not easy to see why this form and extent of liver deterioration occurred in these perch kept at 20°C., for in Experiment 11 (16 days at 28°C.), the prolonged high temperature did not cause any change in the normal

appearance of the liver.

Liver histology in perch from Experiment 12 (7 days at 30°C.) was rather difficult to apprehend. However, in one of four fish examined the liver appeared to contain some necrotic patches, while one other fish had liver cells with misshapen nuclei and some degenerating cell walls with consequent cytoplasm losses (Plate 10, Figure 7).

(vi) Thyroids.

The thyroid tissue is located by section cutting; it lies in the throat region, occurring diffusely around the ventral aorta, but mostly where the first pair of gill arches meet the lower jaw. Sections of thyroid were cut from perch from Experiments 5, 6, 7, 10 & 12.

Insufficient material was examined to secure more than an impression of the effects of temperature on the histology of the thyroid. Short-term effects, as a result of the relatively rapid heating during lethal tests, did not affect the histology of the tissue, but among perch acclimatized to various temperature levels for longer periods it appeared that there was a similar kind of inverse relation of thyroid epithelial height with temperature to that noted for the brown trout by Swift (1959). The appearance of perch thyroid tissue at low temperatures (6-8°C.) was that of colloid-filled follicles

composed of a very low epithelium, the cells of which had oval or bean-shaped nuclei. This cell form is in marked contrast to salmonid thyroid cells, which are much more cuboidal or columnar (depending on the state of the tissue). But they resembled that figured for Astyanax by Rasquin & Rosenbloom (1954). At intermediate temperature levels (Experiment 10) the epithelium appeared flatter still, and at 30°C. (Experiment 12) it was so flat that it appeared merely as a thickish boundary line to the follicles, nuclei appearing as thickenings on this line.

(vii) Axial muscles.

The muscles of the trunk region adjacent to the head kidney were sectioned simultaneously with the latter, and so were routinely examined whenever head kidneys were studied. Plate 11, Figure 1 shows a typical section through normal muscles in this region. Close packing of the fibres is apparent, as is the presence of the fibrils of which the former are composed. Some nuclei are visible on the sarcolemma. While there are parts of such sections through the trunk region where the packing of the fibres is not nearly so close, nuclei are still, in the main, applied to individual fibres, and the fibril structure remains clear.

However, in Experiments 11 and 12 (28 and 30°C.) some of the fish examined showed notable changes in many of the muscle fibres of this region. Not only was there general

Plate 11. Sections through perch axial muscles.

Figure 1. Muscles of low temperature control fish (acclimatized at 7.5°C.). Close packing of fibres and normal fibril structure are apparent. Nuclei may be seen.

Figure 2. Muscles of fish acclimatized 7 days at 30°C. (Experiment 12). Note wide separation of fibres, with loss of structure. See text for detailed comment.

Both X 600.

Plate 11.

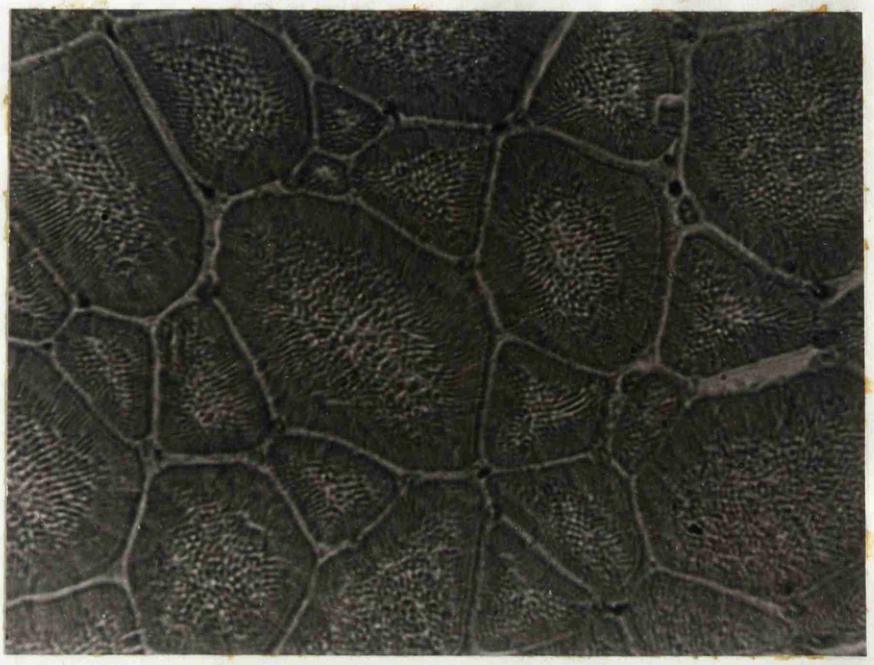


Fig. 1

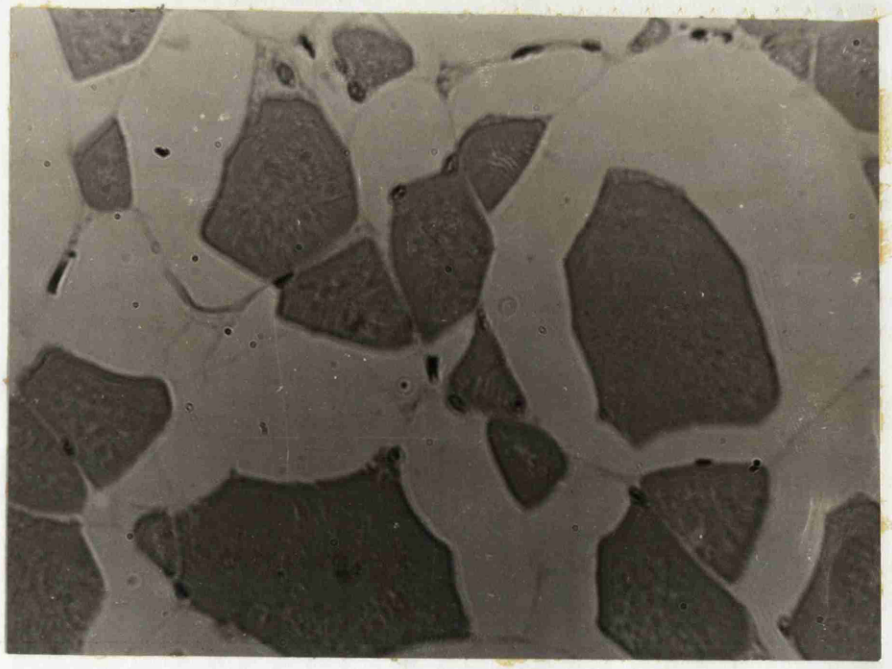


Fig. 2

atrophy of individual fibres, so that these had shrunk from their sarcolemmas in such a manner that many nuclei now appeared to be suspended between fibres, instead of at their usual position at the periphery of fibres, but, in addition, in many instances the fibril structure had degenerated to a condition where the fibre appeared to be almost a solid, homogeneous whole (Plate 11, Figure 2.) Gradations between this condition and one of mere shrinkage could be found in the one section, but the great bulk of muscle fibres in these affected fish were changed in this manner.

(b) Condition Factor (K)

Most of the observations on high temperature effects were histological, but a note on condition ($K = \frac{W\ 10^2}{L^3}$) may be inserted here.

Though Le Cren (1951) has extensively reviewed various methods of calculating and expressing condition, length-weight relationship, ponderal index, etc., with special reference to P. fluviatilis, the present writer is not in general agreement with his views, preferring the simple and traditional ratio indicated above. It would not be pertinent to consider here the several reasons for this preference, but Beverton & Holt (1957) have provided a succinct review of the subject, and the writer is in wide agreement with their conclusions. In any event, the simple "K" factor provided an adequate rating of heaviness per unit length

for a group of fish in which the size range was small, and which were immature at time of measurement: see Table 5.

In the aquarium tanks it had been noted that perch held at 20°C. or more for any considerable length of time began to look somewhat thinner than usual, even though they were fed much more heavily than at the lower temperatures; it will be recalled that normal daily activity at these higher temperatures greatly exceeded that at lower temperatures. Table 5 shows that the condition of perch held at 20°C., and more, was less than at the lower temperatures.

The actual rate at which condition was lost at higher temperatures is not known, nor is the level known at which condition might stabilize again at these temperatures, though visual impressions suggested that condition had, in fact become fairly stable in Experiments 11 and 12 at the time of their termination. Fry (1951) referred to similar condition losses among freely fed speckled trout held at the highest temperature compatible with a continued existence (25.3°C. in the case of that species), and Cocking (1957) reported the same for roach held at high temperatures. Cocking (1959,b) also found that ammonia excretion by roach rises with increasing temperature. This is certainly indicative of increasing protein catabolism.

*TABLE 5.*

*Condition (K) of perch acclimatized to various temperatures*

| Acclimatization Temperature (°C) | 6 - 8 | 17.5 | 17.5 | 17.5 | 20 | 20 | 28 | 30 |
|---|---|---|---|---|---|---|---|---|
| Duration of exposure (days) | Several weeks | 2 | 7 | 18 | 8 | 30 | 16 | 7 |
| Condition (K) | 0.725 | 0.694 | 0.723 | 0.690 | 0.692 | 0.663 | 0.658 | 0.635 |
| No. fish | 37 | 11 | 16 | 18 | 22 | 12 | 12 | 9 |

(c) Discussion.

The fact of overall importance emerging from this study so far is that the interrenal tissue of perch showed a graded change in appearance, increasing with the level of temperature at which the fish were held. But the lability of this tissue is such that even a short-term exposure to higher temperatures (e.g. during the rapid heating of a lethal test) was sufficient to alter the appearance of its cells considerably. Since with exposure to progressively higher temperatures for prolonged periods (of acclimatization), cellular changes were also progressive, it seems reasonable, on an histological interpretation, that the condition of the interrenal tissue noted at, say 30°C., was merely a more advanced stage of essentially the same condition noted at 17.5 and 20°C., or during the relatively rapid elevation of temperature in a lethal experiment.

Of other changes, those in condition (K) were only notable for perch exposed to high temperatures for prolonged periods. But under these condition it was clear, even from visual inspection, that fish had become thinner. This loss in condition is of course correlated with the marked degenerative changes observed in muscles. As Plate 11 showed there was pronounced wasting of muscle fibres coupled with advanced loss of structure.

Loss of lymphocytes by the head kidney seemed to be graded with temperature, though with some indication of an ability for lymphoid recovery if the stress-producing temperature was not excessive, and given sufficient time.

Renal tubules and the cells and livers of muscles displayed responses to prolonged higher temperatures, though not graded responses. Thus in Experiment 12 (30°C.), and to a lesser degree in Experiment 11 (28°C.) many of the renal tubules of perch were more thin-walled than usual, because of cellular atrophy, though the nuclei of the atrophied cells looked normal if somewhat shrunken; however it was not until such higher acclimatization temperatures were reached that renal changes of this kind were seen.

Such changes were evidently insufficient to impair the ability of the fish to carry on a day-to-day existence, and were certainly not of the order of the major tubule changes Rasquin & Rosenbloom (1954) described for Astyanax under stress. Nor was there any resemblance to the "endocrine kidney" or other marked degenerative changes reported for the kidneys of stressed rats by numerous workers (Selye, 1950).

The livers of perch fixed immediately after heat death showed a tendency for tissue disintegration

(apparently occurring during the last few minutes of life), but other, more profound changes were found after exposure to 20°C. for 30 days, and to 30°C. for 7 days, though, strangely, not at 28°C. The former type of change, that of tissue breakdown, seemed to result merely from the tendency for simple separation of one liver cell from another. In this connexion it may be observed that Elias (1955) gave an account, based on studies with the electron microscope, of the way in which liver cells appear to be fastened to each other by structures in their walls resembling snap fasteners. This separation of cells was in many instances followed by a tendency for disintegration of the wall of the cell, leading to loss of cytoplasmic contents. The latter, more profound, liver changes somewhat resembled the fatty degeneration of the liver recorded for stressed Astyanax by Rasquin & Rosenbloom (1954).

The literature does not seem to contain descriptions of anything very similar to the changes observed in muscle cells of some perch at prolonged high temperatures. Selye (1950) described various kinds of degeneration of muscular function in men and rats following exposures to stress, but nothing as structurally notable as in perch.

Rasquin & Rosenbloom (1954) found that pronounced muscle wastage accompanied other symptoms of stress in Astyanax kept in darkness, which certainly seems to be degeneration in the same direction, though from their photomicrographs of sections of these there is not much indication of marked change in appearance of the structure of the fibres.

Of the organs and tissues examined, only the spleen showed no apparent change even after prolonged exposure to very high temperatures. On the other hand, the spleen could always be relied upon, no matter what the thermal history of the fish from which it had been removed, to become considerably depleted of lymphocytes as a result of exposure to lethal temperatures.

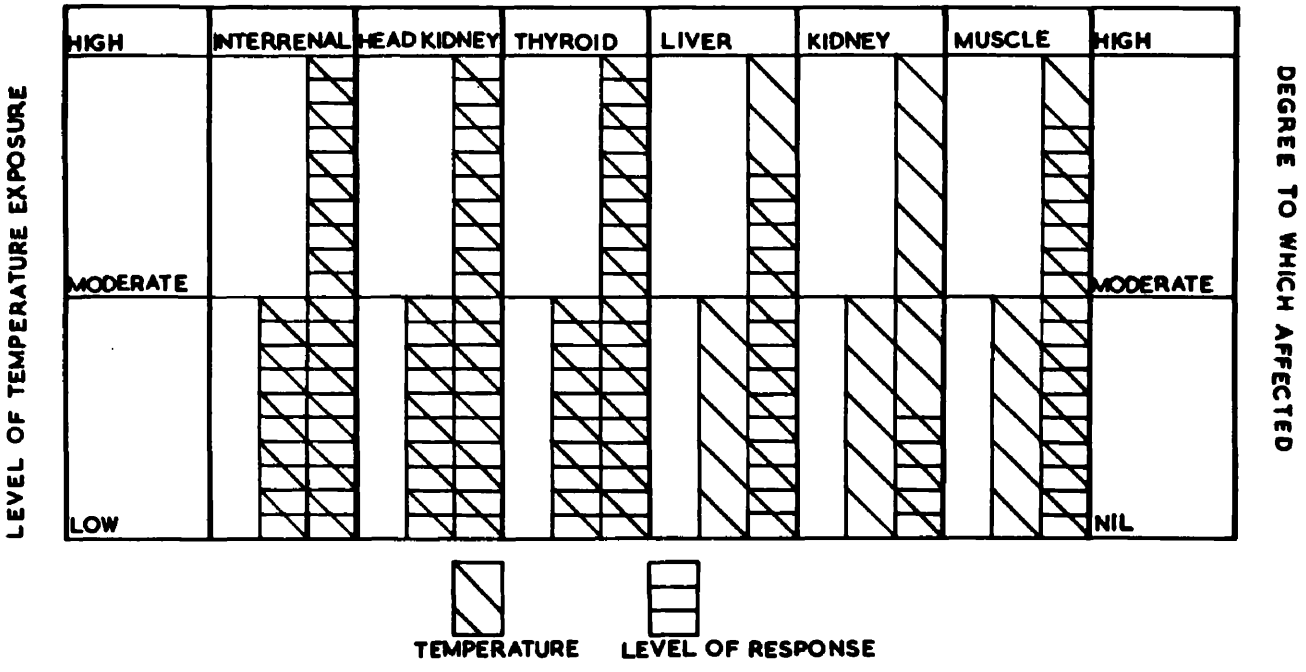
The follicular epithelium of the thyroid, though not extensively studied, appeared to change somewhat at prolonged higher temperatures.

Figure 15 presents in summary the essential points arising from this section of the work. It indicates that interrenal tissue, head kidney and (probably) thyroid, showed histological changes which corresponded to the level of temperature to which the perch were exposed. It is assumed that the change in interrenal

Figure 15. Diagrammatic representation of observed histological changes in Perca fluviatilis following exposure at various levels of temperature.

Responses of interrenal, head kidney and thyroid tissues, manifested as described in Section IV, are closely geared to the thermal background; in this connexion thyroid is not as labile as the others, but appears, nonetheless, to be affected in a graded manner during longer periods of exposure.

Liver tissue is only significantly changed in appearance after the more extreme action of temperature, whether this be a prolonged exposure to some higher temperature, or as a result of a lethal test. Renal tubules (kidney) are only involved during long exposure to very high temperatures, and much the same applies to muscle.


HIGH
INTERRENAL
HEAD KIDNEY
THYROID
LIVER
KIDNEY
MUSCLE
HIGH
MODERATE
MODERATE
LOW
NIL
LEVEL OF TEMPERATURE EXPOSURE
DEGREE TO WHICH AFFECTED
TEMPERATURE
LEVEL OF RESPONSE

tissue which occurs even at moderate temperatures signifies either a direct relationship between temperature and the activity of this tissue, or one in which the pituitary has the role of an intermediary. It will be recalled that this change takes the form both of hypertrophy or atrophy of interrenal cells (depending on circumstances), and at very high temperatures hyperplasia may also occur.

As indicated previously it is considered that blood flow, as influenced by temperature, may be the main determinant of the lymphocyte losses seen in the head kidney following exposure to rapid temperature elevation, or to higher levels of acclimatization. This view is perhaps strengthened by the fact that blood supply to the head kidney is very considerable. The dorsal aorta passes between the lobes, and the organ is drained by the very large cardinal veins which directly traverse its substance.

The writer wishes to avoid making any firm statement about the possible significance of thyroid changes in relation to temperature. As Hoar (1959) has recently pointed out *"thyroid activity in fish has been associated with seasons of rapid growth, sexual development, osmotic changes in the environment and with transformations such*

*as the smolt change in salmonids and the metamorphosis of flatfish".* So far as the relation to the temperature of the environmental water is concerned the findings of various workers appear to be in some contrast to each other. A final note on the thyroid is deferred until the General Discussion (Section VII).

Histological changes in liver, kidney and muscle all occurred at higher levels of temperature when the exposure to such temperatures was prolonged. Though none of the fish displaying these changes seemed superficially impaired in their mobility or general behaviour the changes are seen as essentially degenerative, indicative of widespread incipient organic deterioration of the animal, though they had not progressed far enough to cause actual and obvious distress. It may be that the changes would never have gone much further, or that actual repair would eventually have taken place even in the continued presence of stressful temperatures. But even though not all the fish living at these higher temperatures displayed the lesions the fact that some did is taken to mean that the group as a whole were arriving at a critical point in their adaptive potential, and that just a little more call upon this potential by the thermal environment would have been enough to tip

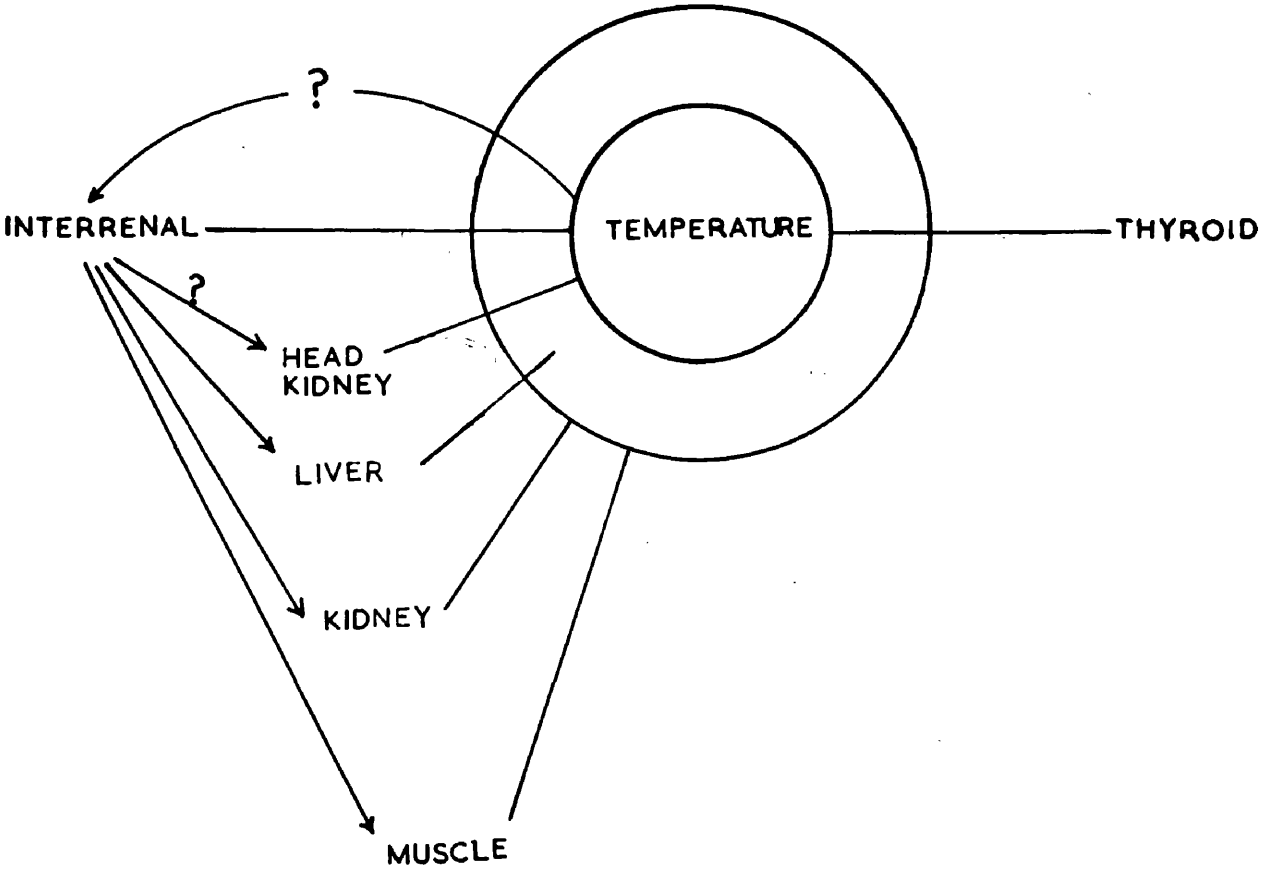
the balance against them, thereby involving more seriously those already affected, and drawing into the affected group those still without obvious histological change.

An attempt is made in Figure 16 to suggest schematically the relations between temperature and the changes wrought in the organs and tissues which have been considered. Because of its position as adrenocortical homologue interrenal tissue has been assigned the probable role of an endocrine organ, and the suggestion is made that it may have affected other organs directly or indirectly. The head kidney is perhaps largely excepted from this concept, because of the suspected importance of blood flow in influencing its appearance, and the spleen has been omitted completely, because its changes take place only in the last few minutes of life before heat death. The thyroid is thought to be separately involved, because its activity is presumably determined by the thyrotrophic hormone of the pituitary.

There would seem to be reasonable grounds for allowing a scheme along these lines, since most of the lesions found in perch are not dissimilar to those found in certain stages of the General Adaptation

Figure 16. This indicates a possible relation between temperature, interrenal activity and damage to tissues. The scheme implies that interrenal secretory rate depends on level of temperature, so when this is low secretory rate is low, and vice versa. The region between the two circles represents the range of levels of thermal exposures used in the present series of experiments. The lines leading from this region to the names of various tissues signify the approximate thermal level associated with histological change. Thus the line traversing the region between the circles and continuing to INTERRENAL indicates that this tissue would appear to be delicately geared to temperature at all levels between 7 and 30°C, to judge from histological appearances. Muscle, on the other hand, is only affected by exposure to high and prolonged temperature, so its line starts from the outer circle. However, it is proposed that the tissue changes occur via interrenal influence, rather than as direct results of temperature, though head kidney may be something of an exception here. The interrogation sign is to suggest that the interrenal itself is probably under the influence of some central control system (the pituitary in all likliehood) on which temperature exerts its primary effect.

The thyroid is probably largely independent of the changes to the left of this scheme, though here also the pituitary is probably an intermediary.


?
INTERRENAL
TEMPERATURE
THYROID
?
HEAD
KIDNEY
LIVER
KIDNEY
MUSCLE

Syndrome described by Selye (1950), while Rasquin (1951) and Rasquin & Rosenbloom (1954) have reported changes apparently rather akin to these in Astyanax exposed to several kinds of stress including cold and darkness.

If there is here a syndrome for teleosts at all resembling that for mammals exposed to prolonged noxious stimuli it should be testable by somewhat similar methods, and the next section is devoted to such tests and their consideration.

The essential points to be investigated further seem to be as follows: Is the effect of temperature on the interrenal a direct one on the secretory (and metabolic) activity of this tissue? since after all the perch is a poikilotherm; or is the interrenal tissue under pituitary control as in mammals? To what extent is the level of tolerance to higher temperatures (as exemplified in lethal temperature tests), and its variation with varying thermal history, affected by the activities of the interrenal tissue?

Justification for this last question arises from a belief that interrenal tissue is a secretory tissue with a function closely akin to mammalian adrenocortical tissue, and it would therefore follow that it may be

involved in the combatting of stress in the teleost body in much the same way as the adrenal cortex is known to be in the mammalian body.

## V. MECHANISM OF TISSUE IMPAIRMENT AND LETHAL EFFECTS ACCOMPANYING EXPOSURE TO HIGHER TEMPERATURES

Pronounced activation of the adrenal cortex under stress is one of the classical attributes of the General Adaptation Syndrome of mammals. The elucidation of this syndrome has shown that cortisone, the active principle secreted by the adrenal cortex, is the main hormone responsible for the numerous systemic and organ changes in the body following exposure to a 'stressor' or noxious stimulus. In mammals the rate of secretion of cortisone is governed by the secretion of an active principle of the anterior pituitary (corticotrophin or ACTH), so that it appears feasible, in interpreting in greater detail the scheme outlined in Figure 16, to suggest that in the perch, too, the pituitary may be the intermediary between higher temperature and observed changes in the interrenal tissue. This view assumes of course that interrenal changes indicate genuine functional changes. Suggestive indirect evidence comes from the studies of Dittus (1940), Rasquin (1951) and Rasquin & Rosenbloom (1954) who showed that the interrenals of certain fishes showed pronounced changes following exposure to stress,

or after administration to the fish of mammalian ACTH.

In all, three procedures suggested themselves as tests of whether ACTH or some closely similar substance is involved in the response of perch to high temperature.

(I) A fundamental feature of the Adaptation Syndrome of mammals is that a great variety of noxious stimuli of very diverse character elicit essentially the same broad changes in the body, and these changes may often be identified through histological appearances of specific organs or regions of the body. If the mortality of fish exposed to high temperature is not to be thought of just as a simple summation of the reactions of various tissues, but as the final breakdown or disfunction of a general systemic response system, whose capacity has been exceeded, then other stimuli or stressors, not fundamentally related to high temperature, should be able to call forth much the same basic response by the organism. Therefore the histology of certain tissues of fish which had been exposed to several levels of cold, oxygen lack, and osmotic load was examined to see whether the changes wrought in any measure resembled those elicited by high temperature.

(2) Others have claimed that mammalian ACTH can change the interrenal appearance in fish, so perch from the aquarium stock tanks were injected with ACTH and also with cortisone, adrenalin, and isotonic saline and compared histologically with uninjected controls from the same stock tank.

(3) In this study high temperatures are regarded as noxious stimuli. If the interrenal tissue be engaged in combatting their effects it follows rationally that it would be both sound therapy and a demonstration of the involvement of the tissue if its activity could be stimulated artificially, and temperature stress thereby relieved somewhat. Experiments were performed to test this line of reasoning, the main criterion for success being whether the lethal temperature was significantly raised as a result of treatment. In these experiments injections were given of ACTH, cortisone, adrenalin, and head kidney and pituitary brein.

(4) A fourth - and obviously basic - method would have involved extirpation of the pituitary, for if the hypothesis of the role of corticotrophin or some similar substance is correct extirpation would have been tantamount to removing the main centre of control for

mechanisms dealing with noxious stimuli. Furthermore, if removal of the pituitary had proved possible it would have been most interesting to discover whether, in the first place, the lethal temperature was significantly lowered by this means, and secondly if such lowered tolerance could be restored by administration of ACTH, etc. However, unlike the pituitary of the minnow, for example, which is readily accessible above a thin layer of bone forming the roof of the mouth, and is guarded by more bone from the brain, the perch pituitary unfortunately lies above a deep ridge of bone against the unprotected optic region of the brain. The surgery required to approach it proved too drastic to permit survival. Moreover, the branches of the dorsal aorta which lie in the way make the cutting of any hole in this vicinity exceedingly hazardous. After numerous attempts to solve this problem it was abandoned - reluctantly since its solution seemed to hold such obvious promise.

The saline control injections given in the experiments described in this Section were based on the osmotic pressure of the serum of P. fluviatilis (Krogh, 1939). This is the approximate equivalent of 0.84% sodium chloride solution, which was the concentration employed.

The length and weight data for the fish used in the experiments described in this section are as follows:-

| | Length (cm.) | Weight (g.) |
|---|---|---|
| Mean | 11.6 | 15.8 |
| Range | 9.7 - 13.5 | 9.0 - 24.5 |

About 68% of them were males.

(a) Stimuli Other Than High Temperature

(i) Osmotic load

The osmotic pressure of perch blood is about one quarter that of sea water. Concentrations of 10, 30, and 70 % sea water were prepared by diluting sea water with aquarium water. One perch, removed from the aquarium stock tank in which it had been for more than a week previously, was placed in each of three glass museum jars containing 2.3 l. of one of the above concentrations of sea water. The water in each jar was aerated from the aquarium's compressed air line. During the experiment the temperature in the jars was 6-7°C., which had also been the approximate range for several weeks in the stock tank.

After 12 hours the perch in 70% sea water was removed and fixed in Bouin's fluid; those in 10 and

30% sea water were removed and fixed after 4 days. A control fish from the stock tank was also fixed in Bouin's.

After one hour in the various concentrations of sea water the three fish looked rather irritable, the one in 70% showed a slight loss of lateral stability and rather spasmodic respiratory movements. After two hours the corneas of this fish had become nearly opaque and after four hours the eyes looked almost completely blind. Though still able to wriggle vigorously when handled this fish had lost nearly all co-ordination, after 12 hours in 70% sea water, and lay helplessly at the bottom of its container. At this point it was removed and fixed in Bouin's. It is worth recording that in the last few hours of its exposure the formerly opaque corneas had cleared again, though the lenses were still clouded.

The two fish in 10% and 30% sea water were, superficially, unimpaired at time of fixation ( 4 days after beginning of exposure).

The histological examination of these three fish revealed the following as compared with the control fish:-

Head kidneys

Considerable loss of lymphocytes had occurred from the head kidneys of the three fish in diluted sea water.

Interrenals

The appearance of the control interrenal tissue was of the usual plump cuboidal or polyhedral cells with granular cytoplasm. Interrenal cells of fish from 10 and 30% sea water looked somewhat "stressed" in the manner associated with thermal exposures described in Section IV. It appeared that initial cellular hypertrophy had occurred, followed by atrophy, since the cells were longer than normal but of wasted appearance. Much cylinder formation was seen, and many blood spaces among the cells. In fish which had been in 70% sea water very marked atrophy was evident.

Renal tubules

All appeared much as normal.

Livers

The greatest difference from the control liver was seen in the fish which had been in 70% sea water. In this the cells had tended to shrink apart strongly from each other, though the nuclei and cytoplasm looked quite

normal and there was no evidence of necrosis. In the fish from 30% sea water the liver had small patches which appeared necrotic, and there was even a slight suggestion of cellular separation in the fish from 10% sea water.

Spleens

The only spleen departing significantly from the normal in appearance was that from the fish which spent 4 days in 30% sea water, in which almost the whole of the lymphocytes had been replaced by erythrocytes, with which the organ was packed.

Thyroids

There was little to note here, save perhaps a slightly thicker epithelium than usual in the follicles of the 70% sea water fish.

Muscles

Only those of the perch exposed to 70% sea water were abnormal. These muscles appeared very similar to those already described for fish exposed to very high temperatures for long periods (Experiments 11 & 12, Section IV). That is to say, there was extreme shrinkage causing separation of the fibres and disappearance of their typical fibrillar structure.

(ii) Low Oxygen

As in the experiment described above perch were placed in jars, which this time were filled completely with aquarium water and then sealed, so that the fish, by their own respiratory activity, lowered the level of oxygen in solution and raised the carbon dioxide level. Table 6 supplies information on these points.

The fish were killed and Bouin-fixed at the oxygen and carbon dioxide values shown in the Table. Subsequently their tissues were examined histologically.

Head kidneys

Moderate losses of lymphocytes appeared in the head kidney of Fish 2 as compared with the control, and there was evidence of very vigorous circulatory activity at the time of fixation, since the blood vessels and channels of the head kidney were packed with erythrocytes. In Fish 3 the position was rather similar, but in Fish 4 the head kidney was severely depleted of lymphocytes so that the reticulum of this organ was clearly discernible.

Interrenals

Even in Fish 2 the interrenal tissue had the appearance of much stress, with marked atrophy and distortion of cells; in Fish 3 conditions were similar. In Fish 4 however,

*TABLE 6.*

*Perch exposed to oxygen lack for histological purposes*

| *Fish No.* | *Temp. (°C.)* | *Oxygen mg/l.* | *Carbon dioxide mg/l.* |
|---|---|---|---|
| *1 (control)* | *9.5* | *10.52* | *2.1* |
| *2* | *9.6* | *5.45* | *7.5* |
| *3* | *9.5* | *2.08* | *14.9* |
| *4* | *7.4* | *0.56* | *14.1* |

The values given were obtained at the end of the experimental period when the fish were removed from the sealed jars and fixed in Bouin's.

atrophy of interrenal cells was extreme. The characteristic tear shapes, reduction of cytoplasm to a mere rim around the nucleus, but with the nucleus itself apparently undamaged, all associated previously with exposure to great thermal stress, were again present.

Renal tubules

Though the control and Fish 2 were as normal in appearance, the tubules of Fish 3 showed, perhaps, hints of deterioration, and in those of Fish 4 there had appeared the most obvious signs of degeneration. Much shrinkage or atrophy of the cells of the tubules was evident, and in some instances cellular disintegration with nuclear extrusion appeared to be taking place (Plate 12).

Livers

In Fish 3 and 4 the livers showed evident impairment. The liver cells were somewhat atrophied, misshapen, and tended to be separated from one another.

Spleens

Except in Fish 4 there was little indication of any departure from normal. However, this fish was moribund when fixed, and slight loss of lymphocytes was apparent.

Plate 12. Renal tubules in perch head kidney.

Figure 1. Degenerating condition of cells of renal tubules in highly anoxic perch. Some nuclei appear to be in the process of being extruded. See text for further explanation.

X 600.

Plate 12.

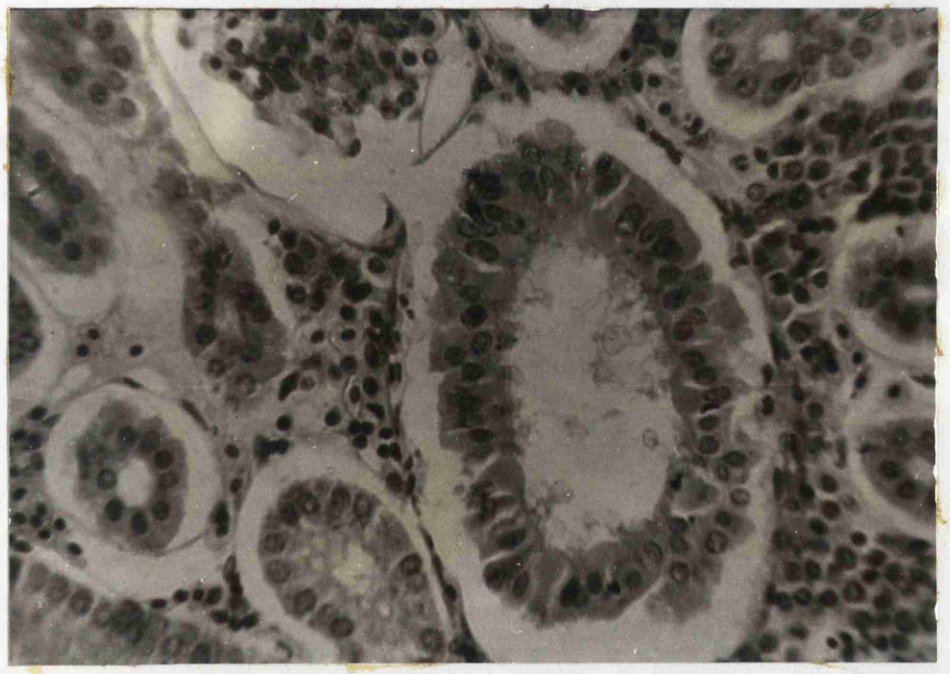


Fig. 1

Thyroids and muscles

No changes detected.

(iii) Low temperature

In this experiment, again in museum jars, 2 fish were cooled down to low temperatures. Fish 1 was lowered over 6 hours from 6.1 to 1.2°C., while Fish 2 was cooled from 7.1 to 0.6°C. in 1½ hours, then held for 11 hours at about 0.5°C. At the end of these periods of exposure to low temperature the fish were fixed immediately in Bouin's.

Head Kidney

Only very slight lymphocyte losses were manifest in either of the stressed fish.

Interrenals

There was noticeable cellular hypertrophy and apparent hyperplasia in Fish 1. There had also apparently been a hyperplasia in Fish 2 but subsequent cellular atrophy had tended to obscure this.

Renal tubules

No changes seen.

Livers

In Fish 2 there was indication of tissue disintegration.

Spleens and muscles

No changes seen.

Thyroids

It appeared that in Fish 2 there may have occurred some proliferation of the actual number of follicles.

(iv) Effects of ACTH (corticotrophin), cortisone, adrenalin and saline.

Five perch acclimatized at about 7.5°C. in the smaller stock tank were used in this experiment. Dosages of substances injected were as follows:-

| | | | |
|---|---|---|---|
| ACTH | 0.05 | I.U. | in 0.025 ml. isotonic saline |
| cortisone | 0.05 | mg. | " " " " " |
| adrenalin | 0.00014 | mg. | " " " " " |

Of the five perch three were anaesthetised and given one each of the above dosages. A fourth fish was anaesthetised and injected with 0.025 ml. saline, and a fifth was neither anaesthetised nor injected. The five fish were placed in an aerated tank for 3 hours after the injections, then killed and fixed in Bouin's. Subsequent histological examination yielded the information which is given below.

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† *A note on these proprietary preparations, on the anaesthetic used, and on methods of injection, is given in Section V (c), which includes a description of the main body of injection experiments aimed at influencing upper lethal temperature.*

Head kidney

Fairly considerable lymphocyte depletion of the characteristic kind was found in the four injected fish, but there was little to choose between them on this account. The uninjected control fish was approximately normal in appearance; a slight degree of depletion had probably resulted from handling.

Interrenals

The interrenal of the ACTH-injected fish showed evidence of hyperplasia with subsequent cellular atrophy; the cortisone-injected and saline-injected fish showed only moderate atrophy, but pronounced atrophy of interrenal cells was present in the fish which had been injected with adrenalin. The interrenal of the uninjected fish had the typical "unstressed" appearance.

Kidneys, spleens, thyroids, muscles

No significant differences from the control appeared in any of these tissues.

Livers

The livers of the fish receiving ACTH and cortisone showed considerable evidence of tissue breakdown and even cellular disintegration.

(v) Experiments of longer duration involving ACTH administration

Other results from these experiments are also reported in Section V (c) in which the effects of ACTH on lethal temperature are described. Here, however, the purpose is to describe the histological effects of ACTH when administered to fish repeatedly.

Experiment A

In the first of these experiments a batch of 16 perch were held in an acclimatization tank at 9.5°C. and injected daily for 10 days, nine fish receiving 0.1 I.U. of ACTH in 0.025 ml isotonic saline per day, the seven controls being injected daily with 0.025 ml. saline alone (see Section V (b) & Table 7, Expt. 4a for fuller details). At the end of 10 days a sample of the fish was fixed for histological examination, and for purposes of comparison an uninjected fish from an adjacent stock tank in which the temperature had been the same, was also fixed. Histological examination provided an interesting picture of contrasts.

Head Kidney

Though the head kidney of the uninjected perch was much as might be expected for a low temperature control fish, viz., a more or less compact mass, in the perch which had

been saline-injected for 10 days there was the clear development of fairly extensive blood channels associated with - even moderate - stress. In the case of the ACTH-injected fish, though some lymphoid depletion was apparent, this was much less marked than in the saline-injected fish.

Interrenals

The cells of the 'saline control' showed a moderate appearance of stress, with some cells rather spindle-shaped or fusiform, but with a good deal of cytoplasm remaining, withal. But the interrenal cells both of the uninjected fish and of the one which had received ACTH were similar to one another, and appeared as would be expected in unstressed fish held at a rather low temperature. They differed from each other through the existence of a considerably hyperplastic condition in the ACTH-injected fish. Not only was its total bulk of interrenal cells much greater, but many cords and cylinders of cells were present.

Kidneys & livers

Appearances were normal in all the fish.

Spleens

Only in the ACTH-injected fish did the spleen seem to have lost some (in particular small) lymphocytes, since much splenic reticulum was visible.

Thyroids

There was perhaps a tendency of the follicular epithelium of the fish receiving ACTH to be thinner than in the other two.

Experiment B

This experiment resembled Experiment A in its essentials, save that its duration was 9 days, that 15 perch were used and that temperature was raised initially from 9.5 to 20°C., then to 23°C., and finally to 25°C., at which it remained for the last 4 days of the experiment. The results of injections on lethal temperature, given this thermal background, are indicated in Table 8, Experiment 8a. Histological examination of tissues of a saline-injected and an ACTH-injected perch, as compared with an uninjected perch from the low temperature stock tank, gave the information recorded below.

Head Kidneys

In this instance only the uninjected fish had head kidney tissue which was of compact appearance. In the saline- and ACTH-injected fish blood channels were present, though not excessive in extent.

Interrenals

The interrenal tissue provided an interesting picture. While in the uninjected fish the tissue had the appearance

characteristic of an unstressed low temperature control, that in the saline-injected fish had the stressed appearance to be expected in perch living at fairly high temperature. But that of the ACTH-injected perch displayed no obvious indication of its thermal background. Certainly the interrenal cells were smaller than they would be at low temperatures, but they were also compact and well-formed, instead of distorted and attenuated which is the usual condition associated with cellular atrophy in this tissue. Moreover the tissue appeared to be hyperplastic.

Kidneys, spleens & muscles

All seemed unaffected.

Livers

The liver of the ACTH-injected fish looked rather as might be expected if this fish had just suffered heat death. The others were normal in appearance.

Experiment C

A third experiment in this group was started fundamentally as a study of the effects of daily injections of ACTH on a group of fish being gradually acclimatized to higher temperatures. It was hoped to raise this batch of fish gradually to 30°C. much in the manner of those in

Experiment 12, Section IV. Moreover, it was hoped to continue the acclimatization upwards very gradually to some still higher temperature, to determine whether the ACTH-injected members of the group could eventually surpass the saline-injected controls in the final temperature they could endure. However, this hope was circumvented when the thermostat failed somewhat and the temperature rose from 29°C., to which the perch had been gradually elevated over the period of one month, and at which they were living apparently in good condition, to 31.1°C. over the course of one day. Since it had taken so long to raise them to 29°C., and this had included the labour of daily anaesthesia and injection of 18 fish, the dead fish were preserved by fixation in Bouin's fluid, in the possibility that they might reveal enough histologically partly to justify the time expended in looking after them. When they were discovered dead in the evening the water temperature had fallen to less than 29°C., but there was no indication, to within about 12 hours, how long they had been dead. It is therefore extremely difficult to assess the effects of the post mortem period at 29-31°C. on the condition of the tissues. But the histological evidence was interpreted with caution, and only the facts that seem incontrovertible are stressed. Four fish were examined. Two of these had

received 0.1 I.U. ACTH in 0.025 ml. saline daily for 28 days; the other two were controls injected just with 0.025 ml. saline daily.

Head kidneys

In all fish except one of the ACTH-injected there was very considerable development of blood channels of the diffuse type previously associated with fish exposed to high temperatures for long periods (Experiments 11 & 12, Section IV (a)(i)). This diffuse channelling was not immediately obvious because the spaces were so completely filled with erythrocytes, denoting very active blood flow at death. In the one fish (ACTH-injected) which lacked this type of channelling, the actual amount of channel development was much the same, it was just that the channels were wider and less diffuse, more easily apprehended at first glance. However, all four fish showed marked lymphocyte depletion in the head kidney.

Interrenals

The interrenal cells were of typically stressed appearance in the two saline controls, with the atrophied and distorted cell forms of such a condition. It appeared that there might have been some hyperplasia in one of the controls, and there was a rather high frequency of cords and cylinders. In both the control fish interrenal cell

margins seemed, in many instances, to be actually disintegrating (though this may have been a post mortem effect).

The interrenals of the two ACTH-injected fish were alike in not appearing misshapen or markedly atrophied in the manner to be expected after prolonged exposure to very high temperatures, with subsequent heat death. A hyperplastic condition seemed probable in one, and was almost certain in the other, in which many extensive cords and cylinders were present.

Renal tubules

While the kidney tubules of the control fish seemed of relatively normal appearance, in the ACTH-injected fish there was a tendency for the type of atrophy previously reported in Experiments 11 and 12 for fish exposed to high temperatures for extended periods.

Livers

It was impossible to draw any real conclusion concerning the condition of the liver in these fish. In the first place all the livers looked, as might be expected, as if they had come from fish which had suffered heat death. Cellular shrinkage seemed to have gone further than would be usual, probably because of long exposure to post mortem high temperature.

Spleens

Here the lymphocyte depletions associated with heat death had taken place.

Muscles

Of the four perch only one (a saline-injected control) had a completely normal muscular picture. The muscles of the other control looked largely like those of other fish acclimatized to temperatures around 30°C. (Experiments 11 & 12), with marked loss of fibrillar structure. The two ACTH-injected fish showed a condition between degeneration and normality, some fibres appearing normal, others showing nearly total loss of structure, while many fibres were intermediate in appearance between these.

While it could be argued that prolonged post mortem exposure to a temperature of about 30°C. accounts for these changes this seems most unlikely in view of the fact that in one of the four fish the muscles looked normal. Indeed the variability of the appearance in the four fish suggests a variable level of muscular degeneration among them accounted for by slight variation in thermal resistance, from one fish to another.

(b) Experiments to offset Thermal Stress.

As previously mentioned, the aim of these experiments was to determine whether thermal stress could be somewhat

alleviated by procedures calculated to render the response system of the perch more resistant to high temperatures. The criterion of success was whether the upper lethal temperature could be significantly raised.

The experiments fall into three groupds: those in which mammalian ACTH was used, those in which mammalian cortisone and adrenalin were employed, and finally those in which an attempt was made to bring about an effect through the injection of pituitary and head kidney breis made from tissues of freshly-killed teleosts.

Most of the relevant information for this series of experiments is given in Tables 7, 8, 9 and 10.

The ACTH employed has the trade name of ACTHAR and is manufactured by the Armour Laboratories. It is a lyophilised substance, readily soluble in water or saline. Cortisone was in the form of cortisone acetate (miscible with water or saline), as prepared by Upjohn of England. Adrenalin, as adrenalin chloride (1:1000 adrenalin), was a Park, Davis product.

Pituitary and head kidney breis were made from freshly-killed fish captured especially for the purpose. The pituitaries were ground in a mortar, then filtered through fine silk net, with minimal washings of saline. The fluid

thus produced looked almost completely clear. The object of injecting teleost pituitaries was, of course, to inject corticotrophin (ACTH) or some similar active principle in an effort to combat stress. Similarly, the reason for injecting head kidney substance was because this organ contains the interrenal tissue with its (supposed) active principle (cortisone?). The finely-pulped head kidneys were simply mixed with a little saline, to thin their consistency somewhat, before injection.

All injections in this series, and those already mentioned in this thesis, were made into the dorsal muscles of the perch, except in Experiment 7a (Table 8) in which they were interperitoneal. Where a series of injections was given the injection sites were alternated from one side to the other and varied from place to place on the dorsal muscles of any one side. The volumes of fluid injected varied among experiments as the Tables show, but were kept as small as was practicable, consistent with dilutions and dosages.

Perch were anaesthetised before all injections with the water soluble anaesthetic M.S. 222. As Gilbert & Wood (1957) have pointed out, this anaesthetic acts quickly, and apparently there is no cumulative effect. The concentration used was 1:1000 aqueous solution, which

is the highest recommended by Gilbert & Wood. However, as the result of a small preliminary test it was decided that the rapid effects on perch of exposure to this concentration were preferable to a longer period at a lower concentration, and the recovery was swift and complete, with no overt after-effects. It is worth noting that in an attempt to reduce extraneous sources of stress, in certain experiments of a preliminary nature not reported here, it was discovered that M.S. 222 could produce lethal effects. Thus it had been though advisable where the temperature in an experiment was being raised progressively during a series of injections, that M.S. 222 should be pre-heated before use to the temperature prevailing in the acclimatization tank. However, it was found that at 20°C. and above perch died rapidly in a 1:1000 solution of the anaesthetic. This finding was not followed up, but it meant that the temperature of the anaesthetic had always to be kept at or below 15°C. (in practice generally at about 4-5°C.), no matter what the temperature in the experimental tanks. As the immersions in M.S. 222 sufficient to cause anaesthesia were only of 1-2 min. duration, this is not thought to have been a very serious objection.

The experimental and control fish were marked on the tails with small, distinguishing cuts while under the influence of the anaesthetic, so that they could be readily identified.

(1) The effects of mammalian ACTH

These experiments may be subdivided into two groups, in the first of which fish were acclimatized to low temperatures and remained there before testing (Table 7). In the second group of experiments the temperature of the water was progressively raised to higher levels, the fish being concurrently injected several times (Table 8).

In Experiments 1a, 2a and 3a the perch, acclimatized to low temperatures, received just one injection of ACTH (or saline in the case of the controls) before lethal testing. In Experiment 1a highly significant differences between mean upper lethal temperatures of ACTH-injected fish (0.1 & 0.01 I.U.) as compared with saline-injected controls were found ('t' test, Fisher, 1954). In Experiments 2a and 3a differences were not significant - here 0.2 I.U. and 1 I.U. ACTH, respectively, were injected into each fish.

In Experiments 4a and 5a fish acclimatized, and held, at low temperatures were given 0.1 I.U. ACTH daily for 10 and 9 days respectively. The mean upper lethal temperatures

TABLE 7.

Summary of experiments on perch injected with ACTH.

| Expt. No. | Details of procedure | Starting temp. (°C.) | Mean upper lethal temp. (°C.) | Significance of difference between means ("t") |
|---|---|---|---|---|
| 1 a | 5 controls, 0.025 ml. saline<br>5 exptls., 0.01 I.U. ACTH in 0.025 ml. saline<br>5 " 0.1 " " " " " "<br>Injections intramuscular, given $3\frac{1}{2}$ h. before beginning lethal test. | 8.0 | 26.10<br>26.84<br>26.82 | t = 6.900; significant at 1% level<br>t = 8.696 " " " |
| 2 a | 8 controls, 0.025 ml. saline<br>8 exptls., 0.2 I.U. in 0.025 ml. saline<br>Injections intramuscular, given $1\frac{3}{4}$ h. before beginning lethal test. | 7.5 | 26.76<br>26.66 | t = 0.298; not significant |
| 3 a | 4 controls, 0.025 ml saline<br>5 exptls., 1 I.U. ACTH in 0.025 ml. saline<br>Injections intramuscular, given $25\frac{1}{2}$ hr. before beginning lethal test. | 10.0 | 27.12<br>27.12 | No difference between means |
| 4 a | 7 controls, 0.025 ml saline<br>5 exptls., 0.1 I.U. ACTH in 0.025 ml. saline<br>Held at approx. 9.5 deg. Cent. for 10 days, intramuscular injections every day; last injections $1\frac{3}{4}$ h. before lethal test. | 9.5 | 27.88<br>27.94 | t = 5.876; significant at 1% level |
| 5 a | 8 controls, 0.025 ml. saline<br>8 exptls., 0.1 I.U. ACTH in 0.025 saline<br>Held at approx. 10 deg. Cent. for 9 days, intramuscular injections daily; last injection $2\frac{3}{4}$ h. before lethal test. | 13.3 | 28.57<br>29.48 | t = 2.689; significant at 2% level. |

TABLE 8.

Summary of experiments on perch injected with ACTH during progressive elevation of temperature.

| Expt. No. | Details of Procedure | Starting temp. (°C.) | Mean Upper lethal temp. (°C.) | Significance of difference between means ("t") |
|---|---|---|---|---|
| 6 a | 6 controls, 0.025 ml. saline<br>5 exptls., 0.01 I.U. ACTH in 0.025 ml. saline<br>5 exptls., 0.1 I.U. ACTH in " " "<br>Raised to 15 deg. Cent. in ½ h., held at this for 18½ hr, then injected intramuscularly as above; after 5 h. more at 15 deg. Cent. raised to 20 deg. Cent., held at this for 19 h. then injected again; injected twice more at 20 deg. Cent. Total of 68½ hr. at 20 deg. Cent. Last injection 3 h. before lethal test. | 8.5 | 31.27<br>31.54<br>31.68 | t = 1.739; not significant<br>t = 2.483; significant at 5% level |
| 7 a | 5 controls, 0.1 ml saline<br>5 exptls., 0.04 I.U. ACTH in 0.1 ml saline<br>5 exptls., 0.4 I.U. ACTH in " " "<br>Raised to 15 deg. Cent. in ½ hr., held there for 18 h, then injected interperitoneally as above; raised to 20 deg. Cent., held abt. 28 h then injected again; held at 20 deg. Cent. a further 19½ h. then injected again 2½ h. before lethal test. Total of 50 h. at 20 dg. Cent. | 8.5 | 31.20<br>30.72<br>31.54 | t = 1.614; not significant<br>t = 1.271; not significant |
| 8 a | 5 controls, 0.025 ml. saline<br>6 exptls., 0.1 I.U. ACTH in 0.025 ml. saline<br>Raised to 15 deg. Cent., held one day, then to 20 deg. Cent., for 2 days, 23 deg. Cent. for 1 day, finally 25 deg. Cent. for 4 days. Intramuscular injections as above daily. Last injection 3½ h. before lethal test. | 9.5 | 33.56<br>33.50 | t = 1.724; not significant |

of these, as compared with controls receiving saline were highly significant, being about 1°C. higher. This difference is as if the ACTH-injected fish had been acclimatized to a temperature about 2°C. higher than the controls (see Figure 2).

The summarized details of these five experiments is given in Table 7.

In Table 8 are shown the results of giving a series of injections of ACTH to perch whose temperature was elevated to progressively higher levels as the experiments proceeded.

In Experiment 6a this procedure led to a significant difference in upper lethal temperature between the fish receiving 0.1 I.U. ACTH and the controls. In the other batch of fish in Experiment 6a (those receiving 0.01 I.U. ACTH) differences were not significant at the 5% level, and the same holds for Experiment 7a where the general procedure was very similar.

In Experiment 8a, too, where the final temperature was prolonged at 25°C., rather than 20°C., as in Experiments 6a and 7a, ACTH did not affect the upper lethal temperature significantly.

(ii) Cortisone and adrenalin

Table 9 shows that cortisone, injected in three different

TABLE 9.

Experiments with perch inject with cortisone and adrenalin

| Expt. No. | Details of procedure | Starting temp. (°C.) | Mean upper lethal temp. (°C.) | Significance of difference between means ("t") |
|---|---|---|---|---|
| 9 a | 5 controls, 0.02 ml saline<br>5 exptls., 0.05 mg cortisone in 0.02 ml. salin<br>5 exptls., 0.025 mg cortisone in 0.01 ml. sal<br>Intramuscular injections 2¼ h. before beginni<br>of lethal test. | 7.7 | 25.80<br>26.20<br>25.52 | t = 1.909; not significant<br>t = 0.146; " " |
| 10 a | 5 controls, 0.02 ml. saline<br>5 exptls., 0.025 mg. cortisone in 0.01 ml. sal<br>5 exptls., 0.05 mg. " " 0.02 " "<br>Intramuscular injections 2 h. before beginnin<br>lethal test. | 9.8 | 28.20<br>27.88<br>27.76 | t = 1.863 is not significant<br>t = 1.634; is not significant |
| 11 a | 5 controls, 0.025 ml. saline<br>5 exptls., 0.00014 mg. adrenaline in 0.025 ml.<br>saline<br>Intramuscular injections ¼ h. before beginnin<br>of lethal test. | 9.8 | 27.96<br>27.44 | t = 1.874; not significant |

dosages, failed to produce a significant effect on upper lethal temperature, though in all instances but one differences were approaching significance. However, the influence of cortisone would appear to be, if anything, unfavourable, except in the single case in Experiment 9a where fish received 0.05 mg. cortisone per injection.

In the one experiment tried, Experiment 11a, adrenalin had no effect of statistical significance, though it appeared to lower upper lethal temperature slightly.

(iii) Pituitary and head kidney breis

Table 10 shows that two experiments were performed with pituitaries and head kidneys of P. fluviatilis and a third with pituitaries and head kidneys from the powan Coregonus clupeoides, specimens of which were obtained from Loch Lomond where it is the most abundant fish.

These experiments were difficult to appraise. To begin with the amounts of head kidney brei were large compared to most dosages of other substances injected, and the brei was of semi-solid consistency. In addition, head kidney administration involved the injection of much foreign protein. It was felt that the large concentration of unabsorbed solid material in the muscles around the injection site must have led to inflammation, and fish injected with head kidney brei certainly looked 'uncomfortable' and stiff in their swimming movements for some time after.

TABLE 10

Experiments with perch injected with pituitary and head kidney breis.

| Expt. No. | Details of Procedure | Starting temp. (C.) | Mean upper lethal temp. (°C.) | Significance of differ: ence between means ("t") |
|---|---|---|---|---|
| 12 a | 5 controls, 0.2 ml. saline<br>5 exptls., 0.2 ml pituitary brei (from 10 perch)<br>5 exptls., 0.2 ml. h. kid. brei " " "<br>Intramuscular injections 2 h. before beginning of lethal test. | 8.1 | 26.08<br>26.12<br>27.12 | t = 0.093; not signi: :ficant<br>t = 1.502; not signi: :ficant |
| 13 a | 5 controls, 0.02 ml. saline<br>5 exptls., 0.01 ml. pituitary brei (11 perch)<br>5 exptls. 0.1 ml. h. kid. brei " "<br>Intramuscular injections 2 h. before beginning of lethal test. | 9.8 | 28.04<br>28.04<br>27.98 | No difference<br>t = 1.563; not signi: :ficant |
| 14 a | Raised to 15 deg. Cent. and held overnight before injections then as follows<br>6 controls, 0.05 ml. saline<br>5 exptls., 0.04 ml pituitary brei (from 18 powan)<br>5 exptls., 0.025 ml. h. kid. brei " " "<br>Temp. then raised to 20 deg. Cent. for 2nd & 3rd days and injections given daily as follows<br>6 controls, 0.05 ml saline<br>5 exptls., 0.025 ml pituitary brei<br>5 exptls., 0.1 ml. h. kid. brei<br>Intramuscular injections, last given 4½ h. before beginning of lethal test. | 7.7 | 29.93<br>30.28<br>28.72 | 0.266; not signifi: :cant<br>t = 0.880; not signi: :ficant |

While the volume of pituitary brei, which was always much more completely liquid, was not as great, except in Experiment 12a, the fish injected with it were always readily identifiable by the rapid appearance of a prominent pale patch on the skin surrounding the injection puncture.

When upper lethal temperature tests were performed the behaviour of perch injected with pituitary or head kidney brei was more variable than for fish receiving injections of purified products. There may be two main reasons for this. First, a reaction between the tissues of the recipients and donors will vary somewhat from one fish to another; second, some fish seemed more incommoded by the sheer bulk of the head kidney injections than others were. Whether these factors really affected the results of the experiments sufficiently to nullify them cannot be certain. However, the fact remains that this group of experiments was felt to be very variable and unsatisfactory as compared with those in which smaller quantities of purified substances were injected.

In Experiment 12a it will be observed that there was a mean difference of about 1°C. between the mean upper lethal temperature for the head kidney-injected fish as compared with the controls. Yet this difference was not statistically significant, as it mainly depended on the

performance of one particularly resistant fish which failed to succumb until a temperature of 28.9°C. was reached, even though the mean upper lethal temperature for its batch was only 27.1°C. (Table 10).

## (c) Discussion

Experiments described in this Section were designed primarily to determine whether a similar set of histological changes in the perch could be produced by noxious stimuli other than heat (i.e. cold, high salinity, anoxia), whether injected substances, particularly ACTH, could activate the interrenal tissue and produce other changes, and finally whether any of several practices would be effective in offsetting the stress of high temperature. The criterion for success in the latter was whether the lethal temperature could be raised significantly.

Certainly the stimuli other than heat produced several remarkable parallels with the thermal effects described in Section IV. Thus lymphocyte depletion of the head kidney was found in perch exposed both to osmotic loads and anoxia. It did not accompany cold stress to any significant extent, but if, as is believed, it is much influenced by rate of blood flow, then this would be likely to be high in perch in water of high salinity, where a considerable rise in respiratory and circulatory activity would be expected to accompany the increased work to be performed in osmoregulation. Blood flow would almost certainly also be high in an anoxic fish, and very likely low in a fish exposed to cold.

In the interrenal tissue both hyperplasia and subsequent cellular atrophy were detected. Certainly atrophy had taken place in the cases of more extreme stress.

Only in the highly anoxic Fish 4 (Section V (a)(ii)) was there important renal deterioration resulting from these other stressors, but the liver showed the appearance already familiar in this tissue from the study on thermal stress (Section IV).

There was only one remarkable splenic change and it differed from other effects on the spleen noted in this study. This occurred in the fish which spent 4 days in 30 per cent. sea water, and here the spleen lost the great bulk of its lymphocytes and at time of fixation was crammed with red cells.

The slight thyroid changes which appeared to have taken place were insignificant and insufficiently clear compared with other changes to characterise them confidently.

It would not, in general, be expected that the degenerative changes found in perch livers and muscles when these fish were given long-term exposures to high temperatures would be duplicated during the comparatively short-term exposures to these other stressors. Nevertheless the muscles of the fish exposed to 70 per cent. sea water for only 12 hours did

remarkably resemble those from fish in Experiments 11 and 12. But this dramatic effect was probably mainly the result of shrinkage of fibres through osmotic loss of water to the outside medium.

It would seem, then, that these other, and diverse, noxious stimuli or stressors, may produce changes in perch tissues surprisingly similar in appearance to those of heat. This argues convincingly for the notion that changes as a result of thermal stress may be merely a general manifestation of broad systemic changes elicited by any of various noxious agents once the adaptive potential of the fish nears its limit or begins to be exceeded.

.....

Lymphocyte depletion of the head kidney is perhaps one of the most sensitive responses to stress in the perch. From many experiments involving handling and injection, it is felt that even quite moderate manual manipulation may apparently produce a modicum of depletion. Injection itself (merely of saline) seems to cause loss of lymphocytes with fairly clear development of channels: Rasquin (1951) and Rasquin & Atz (1952) found much the same for Astyanax head kidneys following saline injection.

Injection of ACTH in one dose seemed to stimulate the interrenal tissue to become hyperplastic, and the individual cells to atrophy. This seems to imply an increased bulk of secretory tissue coupled subsequently with a stepped up rate of secretion, which is also the characteristic picture seen on exposure to pronounced thermal stress and other noxious agents. A tentative hypothesis was advanced earlier that ACTH or an ACTH-like substance (secreted by the pituitary ?) might be responsible for these changes.

A single injection of adrenalin produced marked interrenal atrophy. It is difficult to evaluate this observation for there is not enough background information on its effects, though Chester Jones (1957) has pointed out that it has been proposed that adrenalin might play an important part in the mechanisms controlling secretion of cortisone by the adrenocortical tissue. In this event, its effect might have been specifically stimulatory. However, in view of the excited appearance of perch injected with adrenalin it seems more likely that it simply acted as a non-specific stimulus, eliciting the same interrenal reaction as many other stimuli, a viewpoint borne out by its inability to influence upper lethal temperature significantly. Indeed, if anything, the effects of adrenalin were unfavourable in this regard, suggesting that

the fish receiving it did suffer a slight degree of shock. It is interesting to note further in this connexion that Dr. H.D. Slack (1960, personal communication) has informed the writer that in Tilapia sp. injection of adrenalin resulted in stress, as manifested in the condition of the gonads.

While the effects of cortisone on the interrenal tissue were not significant as compared with saline injections, this is rather what might be expected if cortisone or some similar substance is itself the secretion of the interrenal, for it is ACTH (or some similar activator) which will stimulate the interrenal, not the interrenal's own secretion.

In experiments involving a series of injections of ACTH several illuminating points emerged. At low temperatures the interrenal cells of a fish receiving repeated doses were of similar appearance to those of an uninjected control, and quite in contrast to the relatively stressed appearance of a saline-injected control. But in addition a hyperplastic condition obtained in the fish receiving ACTH. Even more interesting was the interrenal tissue in fish elevated to 25°C. while receiving ACTH daily, for here, though the cells were somewhat smaller than unstressed cells at lower temperatures they were not at all malformed, in complete contrast to the whole of the former experience with this tissue

at higher temperatures, while a hyperplastic condition again seemed evident. In yet a third instance, following a month of gradual elevation of temperature to 29°C. (when accidental mortality occurred), accompanied by daily injections of ACTH, perch had interrenals both hypertrophic and hyperplastic as compared with their controls receiving saline. It is important to note, moreover, that after this extended period of ACTH administration, the interrenal cells did not look atrophied as would have been expected after heat death, following all previous experience. In case it might be thought that this apparent lack of atrophy was an artifact caused by an indefinite post mortem period in water at about 30°C. it should be remembered that the saline-injected control fish, which had a similar post mortem exposure, did show fairly typical interrenal atrophy.

Thus the evidence indicates that mammalian corticotrophin (ACTH) may considerably modify the appearance of perch interrenal tissue, and as further experiments showed, may elevate the upper lethal temperature of fish receiving it. However, the indications are that temperature tolerance can only be increased appreciably, even with the present rather drastic method of intramuscular or interperitoneal injection, if the acclimatization temperature is less than

25°C.

Finally it appears that ACTH or cortisone injection causes atrophy and some distortion of liver cells; this seems to suggest that liver changes in perch due to high temperature, especially those which occur near the death point, take place mainly under the influence of ACTH, rather than under the direct influence of temperature.

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ACTH definitely had some effects in offsetting thermal stress (Tables 7 and 8). The clearest successes were obtained when the fish were at low temperatures prior to testing, and where dosage did not exceed 0.1 I.U. ACTH per injection. Above this dosage it seemed to be ineffective or even harmful, probably through an effect of overstimulation. While a single dose seemed able to raise the upper lethal temperature by a statistically significant amount it was not certain that repeated doses appreciably improved upon this. From the correlated histological evidence it seems that either one, or more, doses affects the bulk and activity of the interrenal tissue. When the idea of repeated injections with ACTH first occurred it was thought possible that prolonged exposure of perch tissues to higher levels of the secretion of the interrenal might render the animal, as a whole, more tolerant to high

temperature than merely a single dose could do. But it is very doubtful whether this possibility was realised.

Quite apart from the statistical evidence for the elevation in lethal temperature following ACTH administration it was very persuasive to observe the perch directly in the order that they died. In the most successful experiments most of the saline-injected controls died, or were seriously disabled, before the ACTH-injected fish were affected. So there was the graphic visual evidence of the ACTH fish left mostly intact, with the controls quite helpless.

Despite the encouraging histological picture discussed earlier it was only in Experiment 6a, among those in which daily ACTH injections co-incided with progressive elevation of temperature, that lethal temperature was significantly altered. It is felt that the stress of repeated injections themselves operated against success in these experiments, since the fish were concurrently undergoing the stress of a regime of progressive temperature elevation. This view applies especially to Experiment 8a, where the perch had 4 days at 25°C.

The effects of cortisone and adrenalin on thermal tolerance were, if anything, mostly negative. Perhaps dosages were unsuitable, though in the case of adrenalin the dosage is thought to have been a 'physiological' one for the

size of the fish, being based on that which Mott (1951) employed for Anguilla. It is felt that the effect of adrenalin merely added to the stress of the fish - to judge from their excitability after injection and the appearance of tissues. Where so little is known of the effects of mammalian ACTH on teleosts there is always a danger that effects produced may be pharmacological, yet incorrectly interpreted as physiological. Often this will mean that the doses injected may have been larger than necessary to produce a valid physiological response. However, Rasquin & Atz (1952) used, considering the weights of their fish, far larger doses of ACTH in making what they regarded as a valid histophysiological investigation of Antyanax. Considerations of parsimony, that is the wish to obtain a significant response while employing very small amounts, coupled with knowledge of the standard amounts of ACTH usually administered to man for therapeutic purposes, determined the amounts used in the perch work (0.1 & 0.01 I.U.). Thus it was hoped to obtain a response by using much less ACTH than Rasquin & Atz (1952) employed, and the amounts which produced positive results did fall in the region of the amounts used in human therapy, making a due (approximate) proportional adjustment for the difference between the approximate weight of the fish used and the weight of a man. Any more detailed analysis of this problem would have required an extensive separate investigation,

but failure of amounts of ACTH greater than 0.01 or 0.1 I.U. to elevate lethal temperature suggested that at least the right order of magnitude had been struck. Much the same reasoning lay behind the cortisone dosages employed.

Now while it is postulated that ACTH stimulates the interrenal tissues into secreting its active principle this may, of course, not be cortisone. Therefore, while ACTH may offset temperature stress via the interrenal, mammalian cortisone itself need not necessarily be expected to have any such effect.

Effects of pituitary and head kidney breis have already been dealt with. It need only be added here that to inject whole pituitaries, or breis made from them, into a fish is very different from injecting simply ACTH, even if the former contains the latter. As with all teleost pituitaries (Hoar, 1957; Pickford & Atz, 1957) the perch pituitary is a complex organ of many (often uncertain) functions (Kerr, 1942), but capable of secreting many hormones. Some of these very probably control the interrenal's secretions, but to administer a brei of the pituitary is to add all that it contains, while destroying the integrity of the pituitary as an organ, which gives it its power of control. Many of the hormones injected

may well serve to offset the influence of an interrenal-specific hormone, whether ACTH or another. It is felt in retrospect that it would have been a sounder procedure to make implants of whole pituitaries.

# VI. NOTES ON THE HEAD KIDNEY AND KIDNEY OF THE PERCH WITH SPECIAL REGARD TO THE BLOOD VASCULAR SYSTEM

## (a) Methods

There seems to be no detailed account of the anatomy of the head kidney and kidney of adult (or anyway post-larval) P. fluviatilis in the literature. Ströer (1932) gave a brief report of the development of the pronephros in this species, and Baecker (1928) described the head kidney of the adult, though its form is difficult to visualise from his account.

The head kidney and kidney were studied by the examination of serial sections and by the dissection of numerous fish. Injection of the vascular systems of fresh specimens with Indian ink was one of the main techniques employed, coloured latex proving relatively unsuccessful. However, it was often possible to distinguish even the finer branchings of the venous system in the kidneys by direct examination under the dissecting microscope, provided that suitable illumination was used. To this end, it was found useful to direct the beam of a microscope lamp on to the outside of the fish body, somewhat dorsal to the kidney, when sufficient diffused light was transmitted through the muscles of the fish itself to show up the venous

system very effectively. This technique was also effective when ink-injected arterial systems were being examined.

(i) Head kidney

As indicated in Section IV the head kidney in teleosts is apparently derived from the pronephros by replacement with a mass of lymphoid tissue. Figures 17 and 18 depict its characteristic shape in the post-larval perch. The organ consists of two curiously shaped lobes joined postero-medially by a thin bridge of the same lymphoid tissue which also forms the main substance of the organ. The anterior part of each lobe is somewhat lozenge-shaped and is marked off from the body of the lobe by a constriction. Behind this constriction each lobe broadens at the base, while bearing a ridge, which slopes upwards and backwards, along its middle. Each lobe bears a wing-like postero-lateral outgrowth of lymphoid tissue, directed slightly downward. All these features may be made out in Figure 17. The shape is better appreciated if it is realised that the oesophagus or anterior part of the stomach fits snugly between the lobes, and is closely applied to the bridge joining them. Ventrally, the heart is partly accommodated in the space created by the constriction separating the anterior from the posterior parts of each lobe.

Figures 17 and 18 depict the blood vascular systems

Figure 17. Stereogram of perch head kidney from front. Sections through the right lobe are enlarged to show structures typically encountered as the organ is traversed in an antero-posterior direction. These structures lie within the more or less dense lymphoid mass comprising the bulk of the head kidney. The wing-like postero-lateral processes of the organ seem to be composed almost entirely of lymphoid tissue and lack renal structures and blood vessels.

RAC, LAC, signify right and left anterior cardinal veins respectively; RPC, LPC, right and left posterior cardinals; DA, dorsal aorta.

Sectional views

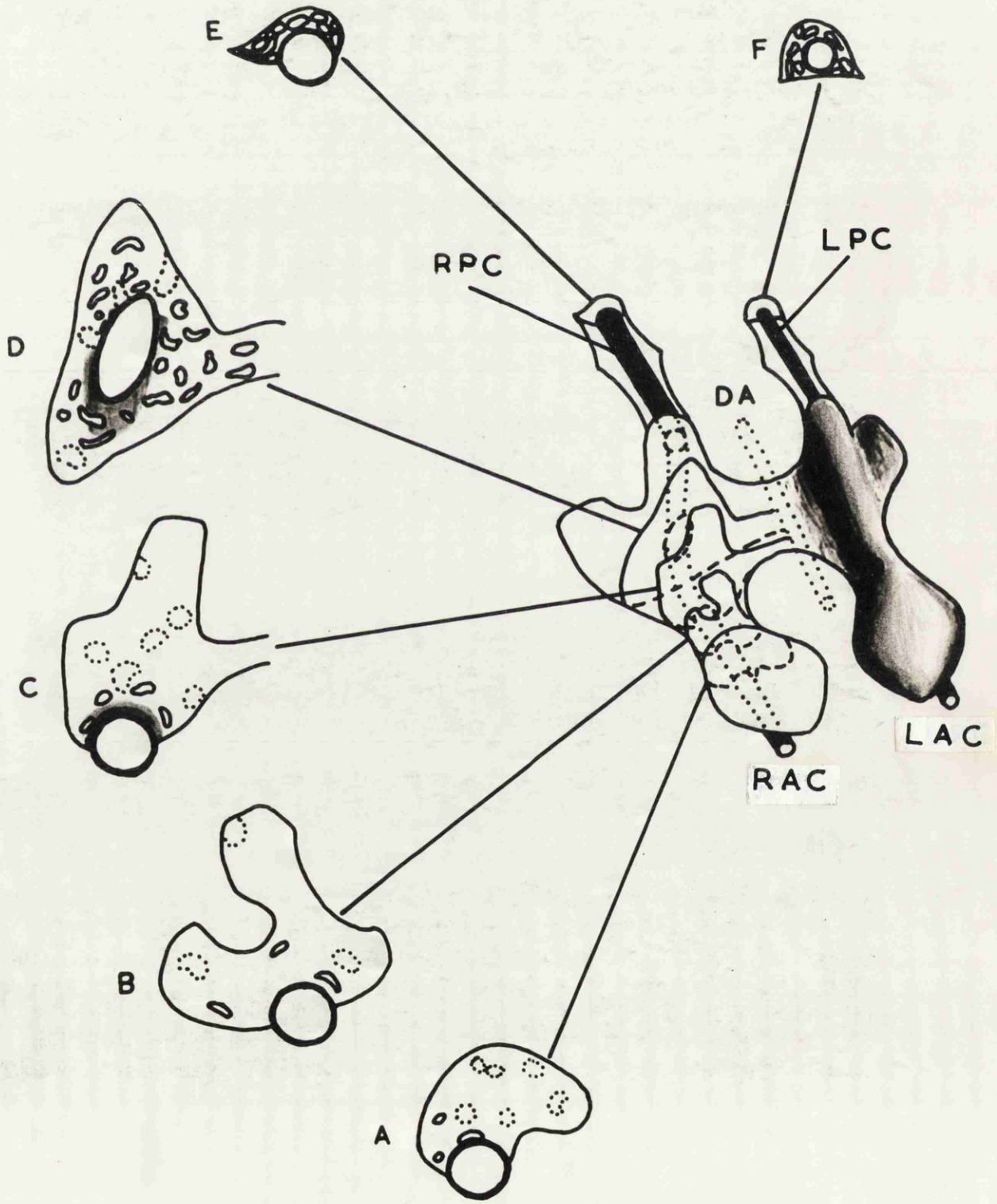
A. Right anterior cardinal vein runs half-embedded ventrally in head kidney. A few renal tubules or corpuscles are present (solid line), and some blood vessels (dotted), all within the matrix of lymphoid tissue.

B. Much as for A.

C. Much as for A, though here there may be a thin layer of interrenal tissue (shaded) around that part of the lumen of the anterior cardinal which lies within the head kidney.

D. The bulk of the interrenal tissue (shaded) occurs here. Renal tubules and corpuscles are also plentiful.

E & F. These are sections through the right and left kidneys respectively. The kidney is essentially a mass of renal structures (especially tubules), with the posterior cardinal half-embedded in it in the case of the right kidney, and running wholly within it in the case of the left. Note the difference in bore between right and left posterior cardinal veins.


E
F
RPC
LPC
D
DA
C
LAC
RAC
B
A

of the head kidney and kidney. The cardinal veins, which traverse the substance of the former, drain into the sinus venosus via the common cardinal. The anterior cardinals run embedded in grooves on the ventral surfaces of the head kidney lobes for somewhat more than half the length of the latter. Each anterior cardinal then enters its lobe, curving upwards and slightly inwards. Within the head kidney it is met by the corresponding posterior cardinal vein descending from the level of the kidney. Together they form an S-shaped figure, and at their junction they are drained ventrally, through the common cardinal, into the sinus venosus.

The dorsal aorta apparently gives off very short and slender connexions to the head kidney (Figure 17 shows its course relevant to this organ). It was not possible to distinguish these branches from epithelial and connective tissue strands in this study, and they could not be injected. Their presence is inferred from the presence in the head kidney of numerous capillaries.

As mentioned earlier the head kidney is essentially lymphoid - a dense mass of closely-packed lymphocytes in a very slight matrix, interpenetrated to a greater or lesser extent with blood channels (the extent being found to vary with thermal background in the present study). The numerous

blood vessels within the lymphoid tissue are readily seen in section, their sectional shape depending, of course, on the plane in which they were cut.

As Figure 17 shows, renal convoluted tubules were present in the head kidney. Renal corpuscles were also present, but, of course, appeared much less often. These renal elements were found through practically the entire length of the head kidney, though they were very infrequent in the most anterior parts of the lobes. The greatest density of tubules and corpuscles occurred well back in the body of the lobes, especially where the anterior and posterior cardinal veins join. Here the tubules are large, often appearing considerably folded in section. These tubules have brush borders. This region of many renal elements is also richly vascularized.

The presence of interrenal tissue around the lumina of the cardinal veins in the head kidney has already been described, together with an account of the appearance of these cells. Figure 17 indicates that the interrenal tissue first makes its appearance in the head kidney (other than for a few isolated patches of cells) where the tissue bridge joining the two head kidney lobes begins. Here it forms a thin layer, against the lumen of the anterior cardinal, a few cells, or even one cell, thick. It appears

only around that part of the cardinal embedded in the head kidney. Furthermore, it must be understood that it does not by any means appear in this region in the majority of perch; but it may be found here. Further back, especially where the anterior cardinals bend upwards and join the posterior cardinals, the greatest quantity of interrenal tissue is found; here the piling up of cells described by Baecker (1928) makes its appearance. This is the invariable region of its greatest abundance. The main accumulation, as Figure 17 suggests, often occurs mostly on the ventral side of the lumina of the cardinal veins.

(ii) The kidneys

The kidneys are slender and run the full length of the body cavity. In section, they are flat ventrally and convex dorsally (Figure 17). The kidneys become united medially about halfway along their length, and as Figure 18 shows they are segmental in form both anterior and posterior to this union. Internally, in contrast to the head kidney, the great bulk of space is given over to renal structures, especially collecting tubules, of more or less circular cross section; the lymphoid elements are reduced to a minimum. Baecker (1928) stated that the united kidneys were thickened at their posterior end through accumulation of lymphoid tissue at that location. No such thickening was ever encountered in the many perch kidneys examined in this study, and examination of sections

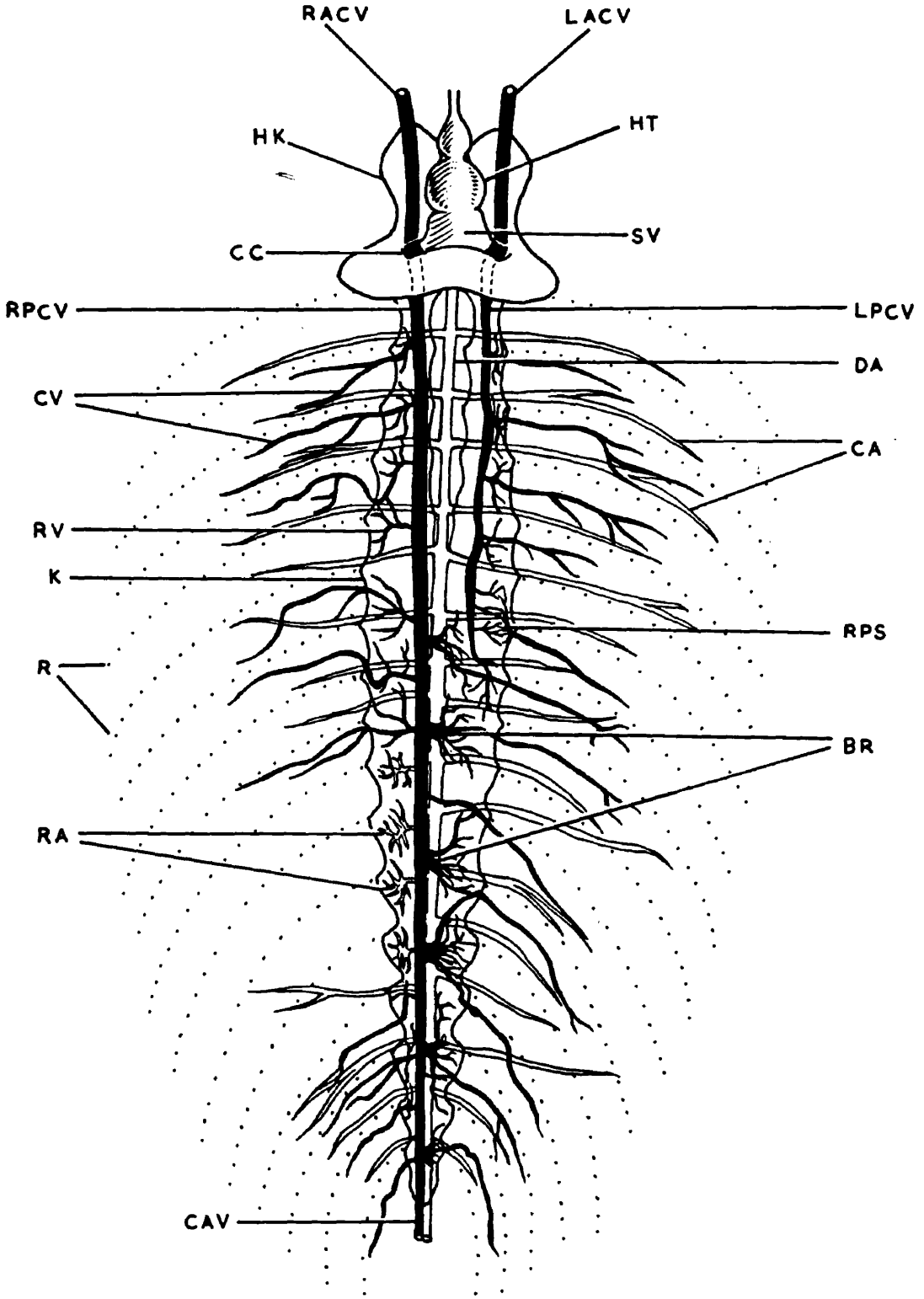
through this area revealed no deposit of lymphoid tissue.

The segmentation of the kidneys corresponds to the spacing of the ribs, and the arrangement of the kidneys' vascular system tends also to follow this arrangement, but only rather vaguely. Thus when one first examines the arterial and venous systems it is noticed that most of the larger costal arteries branching from the aorta have an accompanying vein; but the pattern is variable; not all the segments have a large costal artery and the large veins are much less regular in distribution and disposition than the arteries. Figure 18 suggests more order and regularity than usually exists; it has been made semi-diagrammatic and synoptic in the interest of clarity. The main costal arterial branches depicted merely traverse the kidney substance, without giving off any smaller branches to the kidney itself. Some of these costal arteries follow the line of the rib well up the inside of the body wall, branched, if at all, only simply for the greater part of their course. However, especially towards the posterior end of the kidneys, some of these costal arteries tend to run straight, crossing several ribs in so doing, rather than following closely the line of the ribs. Other main arterial branches may often diverge from the costal arteries close to their bases to supply the dorso-lateral muscle masses, or vertebral arterial branches

Figure 18. Vascular system of head kidney and kidney of perch (semi-diagrammatic, ventral view).

BR, branches of right posterior cardinal vein draining left kidney in lieu of degenerated left posterior cardinal; CA, costal arteries; CAV, caudal artery & vein; CC, common cardinal; CV, costal veins; DA, dorsal aorta; HK, head kidney; HT, heart; K, kidney; LACV, left anterior cardinal vein; LPCV, left posterior cardinal vein; R, ribs; RA, renal arteries; RACV, right anterior cardinal vein; RPCV, right posterior cardinal vein; RPS, renal portal system; RV, renal vein; SV, sinus venosus.

For simplicity the renal arteries appear only at the lower left of the drawing, and all other blood vessels have been omitted from this region.


RACV
LACV
HK
HT
SV
CC
RPCV
LPCV
DA
CV
CA
RV
K
RPS
R
BR
RA
CAV

may turn steeply upwards, their main function apparently being to supply the dorsal muscles. It was not, of course, possible to depict these latter two sorts of arteries in Figure 18, in which only the costals are shown. But, anyway, branches of these three main types may often be given off from one main arterial branch (see also Holstvgood, 1936). Once again it may be stressed that such arteries appear to be functionless as far as the kidney itself is concerned, merely passing through it on their way to the muscles. However, the function of such arteries as regards the kidneys apparently various among teleosts (Holstvgood, 1936).

The renal arteries were revealed by injection as short, simple branches of the aorta which directly enter the substance of the kidney, branching profusely and narrowing progressively therein. They are much less noticeable and of narrower bore than the larger arteries described above.

So far as the venous side of the renal circulation is concerned the right posterior cardinal vein runs the whole length of the head kidney as a large, obvious structure. It is closely applied to the ventral aspect of the kidney, though not deeply embedded.

By contrast, the left posterior cardinal vein narrows on leaving the head kidney and is deeply embedded in the kidney. But the left posterior cardinal terminates at about the seventh or eighth segment, when the right cardinal assumes the task of draining the left as well as the right kidney. The branches performing this function pass below the dorsal aorta as quite thick venous trunks from the right posterior cardinal vein. Figure 18, depicts these features.

There are curious connexions between the veins draining the kidneys and those coming from the body wall; i.e. between the renal and costal veins. Thus while the costal veins may empty directly into the posterior cardinals, simply traversing the kidney structure on the way, they may alternatively join one of the main trunks of the renal veins, or they may break up into a network of venules on entering the kidney to make connexions with the fine branches of the renal veins. The latter of these two kinds of connexions thus constitutes a renal portal system (Figure 18).

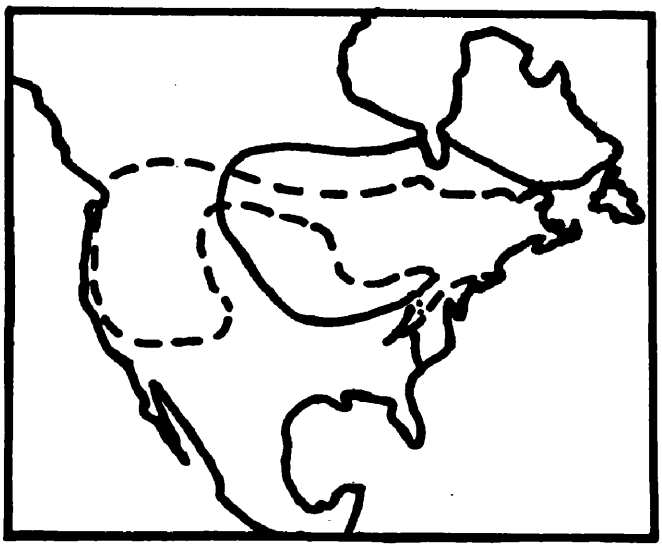
## VII. GENERAL DISCUSSION & CONCLUSIONS

In this study an attempt was made firstly to illustrate that the influence of high temperatures on Perca fluviatilis and its close relative Perca flavescens is of real importance in nature and therefore constitutes a valid problem for further investigation. To this end the approximate range of unfavourable temperatures was established as 29-32°C. for P. fluviatilis and it appears from published data that it is much the same for P. flavescens. A zoogeographic appraisal of the two species then suggested that there are at least two large river systems in the world - in North America and in Australia - where higher temperatures do probably limit the spread of perch towards the equator. It is believed that North America furnishes the best example of the influence of the two primary factors that have been identified as moulding perch distribution. These two factors are, of course, high temperature and steep stream gradient.

In order to underline, finally, the importance of these two factors and the way in which they can so profoundly influence distribution Figure 19 contrasts the distribution of P. flavescens with that of Salmo trutta the brown trout - an introduced species in America. The brown trout inhabits many of the high altitude regions denied the perch (i.e. the Rockies and the Appalachians). On the other hand this species is absent from the main lowland river system of the

Figure 19. The differences in distribution between Perca flavescens and the brown trout Salmo trutta in North America, based on maps and data from Trautman (1957). ——— P. flavescens, ----- S. trutta, limits of distribution. As is obvious the brown trout live in some of the very places barred to perch, such as the Rockies and the Appalachians. On the other hand, the major part of the Mississippi-Missouri-Ohio system is uninhabitable for trout, mainly because of high summer temperatures, lack of suitable spawning facilities, nursery streams, etc.

This is intended to show how a contrast in life habits and thermal tolerances between two species has led to differences in distribution. See text for further information.



Middle West frequented by the perch (i.e. the Mississippi-Missouri-Ohio system), except possibly for some of its highland reaches. Now the habits of brown trout contrast markedly with those of perch. The former is a strong-swimming species, passing much of its existence in swift-flowing highland streams especially necessary for its spawning activities, and thus essential for the completion of its life cycle. The reverse is true of the perch. However, the perch's superior thermal tolerance (upper lethal temperature of brown trout seems to be about 24°C., (Trautman, 1957)) allows it to inhabit much of the Mississippi-Missouri-Ohio system, from which the brown trout is barred. It is hoped that this demonstration, of how opposed tendencies in the biology of these species has resulted in their occupying almost mutually exclusive habitats, will be convincing.

Now both laboratory and field experiences have shown perch as capable of remaining alive indefinitely at temperatures close to their thermal limit. It is concluded that it is probably by a process of thermal adaptation rather than temperature selection that these two Perca species are normally able to avoid heat death. But this means that in certain large river systems those members of a perch population nearest the equator may well be in continual

danger of suffering heat death in midsummer. It may be asked therefore why large, even mass, mortalities are not seen at this time of the year. Possibly the answer is that they might be seen if population density were sufficiently high. However, if the effects of high temperature are insidious, carrying off a few fish at a time, rendering others, through maladaptation, prone to other kinds of mortality than deat death, then many fish might die in summer without ever being accounted for. Moreover, it will only be the comparatively few fish whose environment is at the critical temperature that die. Those a short distance away, in water perhaps only fractionally cooler, will survive. It is argued that for perch something like this must obtain, to produce the recorded distribution in relation to temperature.

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What sort of theory may be inferred from the observations on tissue impairment etc., to account for the effects of high temperature?

The theory which is tentatively advanced is one fashioned in simplified imitation of the General Adaptation Syndrome of Selye (1950). This syndrome, as stated in the

Introduction, draws attention to the similarities of response which occur in the mammalian body as a result of exposure to numerous diverse noxious stimuli or stressors (e.g. heat, cold, post-operative trauma, mechanical crushing, prolonged violent exercise, emotional shock, starvation, etc.). Selye (1950, 1952), in a general account of his theory, attempted to demonstrate the over-riding significance of the anterior pituitary, and through it the adrenal cortex, as controlling agents in eliciting these symptoms. He claims that the active principle of the adrenal cortex (cortisone in mammals) is secreted under the influence of the pituitary, following a sustained stimulus. Cortisone has as its function the maintenance of general physiological and histological integrity in the face of an *"alarming stimulus"*. Selye sees the establishment of a metabolic disorder, or a breakdown of some of the architecture of the body, as what he terms a *"derailment"* of the normal defence mechanism of the body, among which the action of cortisone occupies a key position.

On this interpretation it would seem that cortisone (or the active principle of fish interrenal tissue, whatever it may be) is critically involved in preserving the integrity of the tissues against stressors. If damage, or impairment of function, eventually follows application of a

stressor, then it should probably be considered that the secretory activity of the interrenal has been unable to maintain the level required to preserve integrity. Alternatively it may be that the resistance threshold of the tissues have been lowered through prolonged exposure to stress, despite continued interrenal activity. No firm answer to this problem can be forthcoming at this level of knowledge, though it does appear that ACTH is fundamentally involved in activating the interrenal tissue, and by its injection the thermal resistance of a fish may be raised somewhat.

The question arises of the ultimate importance of the interrenal tissue in controlling thermal resistance. It is certainly not held to be the only factor at work. The vulnerability of the central nervous system as a weak link in the failure of the system as a whole during heat exposures is still unassessed. Although not reported here in detail, it was possible in many instances during the inspection of other tissues to note the condition of sections of brain and spinal cord of perch. Gross or obvious lesions were never observed, even in instances of extreme thermal stress, and as far the methods employed permit it, it can be stated that the histological integrity of the central nervous system was preserved. It is felt that data on the perch thyroid were far too inadequate to form any firm impression

of its function in relation to higher temperatures. Though thyroid cell height is generally accepted as an index of the state of activity of the gland, the relation of this height to temperature appears to vary between fish species. Barrington & Matty (1954), working with minnows Phoxinus phoxinus, found that, while there was a cycle of thyroid activity for this species in nature, it does not appear *"that it is a direct response to temperature"*, but is rather to be correlated in some manner with the reproductive cycle. However, in aquarium experiments, these writers did find that cell-height was positively correlated with temperature, albeit, as they pointed out, the effect was not large. In perch the effects of temperature appeared to be in the reverse direction, that is cell heights (and activity ?) were less at higher temperatures, as Swift (1959) claims for the brown trout. Swift interprets his findings *"as further evidence that the basic function of the thyroid is concerned in the control of the animal's metabolism, in this case in such a fashion as to compensate for changes in the environmental temperature"*. This view appears to have been foreshadowed by a tentative hypothesis put forward by Barrington (1952). Hoar (1957) has pointed out that some fish species show no apparent thyroid response to change in environmental

temperature and has suggested (Hoar & Robertson, 1959) that photoperiod may influence thyroid activity, and through it thermal tolerance.

Pronounced depletions of lymphoid storage organs are prime symptoms of stress in mammals (Selye, 1950). Such depletions occurred regularly, and sometimes spectacularly, in the perch in the face of stress. Yet perhaps the parallel should not be pressed too closely. Selye describes the complete disintegration of many of the cells of such organs as the thymus and spleen, picturing this as beneficial to the organism because *"it may be of great import by supplying special materials needed for systemic defense (e.g. antibodies, activators of the reticulo-endothelial system)"*. Rasquin (1951) and Rasquin & Rosenbloom (1954) also reported much the same response as that typical of stressed mammals for Astyanax given injections of ACTH or cortisone, pituitary implants, or kept in constant darkness. But in the perch head kidney this kind of destruction of lymphoid tissue, with consequent presence of cell debris clearly visible in that organ, was not encountered; furthermore, it was believed that the main cell losses were, fundamentally, an accompaniment of increased blood flow. However, it is quite possible that the erosion of lymphocytes from the head kidney was

effected partly through interrenal activity, increased secretion leading to "fragility" of the lymphoid tissue so that cells were more easily displaced from the reticulum and swept away into the general circulation.

Except for the interrenal changes and the lability of the liver at the thermal death point (plus the odd changes in that organ at the only moderately high level of temperature of Experiment 10) marked changes in response to heat of muscle, liver, thyroid and renal tubules only occurred at high and prolonged temperatures. These changes are similar to those appearing during the advanced stages of the General Adaptation Sundrome. When this similarity is coupled with the sensitive responsiveness of the interrenal tissue and head kidney to several diverse kinds of stress, plus the effects of ACTH on the interrenal and its ability to counteract thermal stress, it really appears likely that the response of the perch to high temperature is essentially an example of the deterioration of a general response mechanism of this teleost remarkably similar to that in mammals.

It is likely that this response system also applies to other teleosts. Rasquin (1951) found that carp pituitary implantation or ACTH injection produced interrenal hypertrophy and hyperplasia in Astyanax mexicanus. In addition, the head kidney was greatly depleted of its

lymphocytes by these treatments, in a manner rather similar to that in perch. As a result of cold stress Rasquin found that in the interrenal tissue *"The amount of cytoplasm was greatly reduced. The cells had shrunk away from each other so that spaces appeared, sharply demarcating them from one another."* This would serve as a good description of the atrophy following thermal stress in the perch - and indeed the other kinds of stress (including cold) to which they were subjected. Rasquin & Rosenbloom (1954) continued this work on Astyanax, to include an histological study of fish kept in continuous darkness as a stressor. Here, too, there were changes in the interrenal tissue, lymphoid depletion and, in more advanced cases of stress, changes in muscle, liver, thyroid, renal tubules and other tissues. Rasquin & Rosenbloom considered that muscle wastage and fat accumulation in the tissues of stressed Astyanax involved an endocrine imbalance in which activity of the interrenal featured prominently. They seem to believe that stress in the first instance suppresses the secretion of growth hormone by the pituitary, and this is probably true since growth ceases. Growth hormone promotes protein build up and fat oxidation, so in its absence they feel the catabolic tendency of interrenal tissue - supposing it to be very

similar in this function to the mammalian adrenal cortex - caused protein breakdown in the muscles. The present writer is unable to follow clearly how Rasquin & Rosenbloom attempt to account for fat storage, but they see it as reciprocally related to muscle protein catabolism. Certainly in the perch, acclimatization to very high levels of temperature resulted in muscle wasting and loss of structure, but this was unaccompanied by any marked fat storage in other organs and tissues. However, the very high activity of perch at high temperature may help to account for this latter fact. Cocking (1957, 1959,b) also found that increased ammonia excretion and correlated weight loss, indicative of elevated muscle catabolism, accompanied exposure of roach to high temperatures. It is felt that the stunted size of P. flavescens associated with life near their thermal limits in nature (Trautman, 1957) is consistent with a curtailment of growth during the heat of midsummer, and is probably related to these phenomena of growth cessation and muscle wastage seen in the laboratory.

In an attempted interpretation of the effects of dietary fats of different iodine values and melting points on thermal resistance Hoar, with two colleagues, (Hoar & Dorchester, 1949; Hoar & Cottle, 1952,a) was able to show

that diets rich in fats with different values of the above two properties could indeed modify the temperature tolerance of goldfish Carassius auratus. But the mechanism for this change is obscure, and the investigations failed to bear out either of the early suppositions of Hoar & Dorchester (1949) that it was degree of unsaturation or the melting points of the fats which was important. However, Hoar & Cottle (1952 b) did point out that the water content of goldfish tissues was directly proportional to the temperature at which they were maintained. They inferred that this is probably because of the increased tendency for water to enter the tissues at high temperatures. Though water content values are not available for perch, from the appearance of the muscles at high temperature, it seems very likely that their water content would be high.

.....

A few last points are worth mentioning. The first is that the location of interrenal tissue, which surrounds the lumen of the cardinal vein in the proximity of the common cardinal, is such that it is admirably located as a secretory organ to release its active principle directly into one of the main venous trunks very close to the heart. This means that its

distribution throughout the body will be both rapid and efficient.

The second point concerns the cylinder formation in the interrenal tissue. Selye (1950) has described the presence of "tubules" in the adrenal cortex which appear to resemble the interrenal cylinders rather closely. He indicates that these are most common in *"the adrenals of animals exposed to very sudden intense stress"*, and certainly they are seen under such conditions in perch, though they are by no means restricted to fish which have suffered stress. Selye considers them to be mainly the result of cytolysis following a mildly hyperplastic condition. In this he may be correct, but the evidence for the mode of formation of the cylinders in the perch is not clear, and their homology with tubules in mammals is not entirely definite.

Cocking (1959a) found the heart still beating in specimens of Rutilis examined after heat death, and the present writer found the same in numerous perch. Cocking proposed that the heart had probably been functioning abnormally before death, despite its continuing to beat afterwards, and that it was therefore inefficient at a time when anything below maximum circulatory efficiency would be likely to lead to anoxia.

Lastly it may be noted that though it was not reported in any detail an attempt was made to determine something of the ability of the interrenal tissue to recover following exposure to thermal stress. This was done by heating perch from an acclimatization temperature of approximately 6°C. until just before disablement (26°C) at the usual rate of 4°C. per hour. They were then cooled down at the same rate to 6°C. again. Ten days later they were fixed. On examination their tissues looked much as would be expected for fish acclimatized to low temperature. So the inference may be drawn that perch may recover completely from even severe temperature stress.

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## IX. APPENDIX.

### Table 1.

Compositions of "Lassie" & "Bemax".

**Lassie** *

| Constituent | Percentage composition |
|---|---|
| Whale meat | 30 |
| Cow meat & offal | 25 |
| Beef liver | 5 |
| Ground fresh bone with adhering meat & marrow | 5 |
| Cereal | 20 |

Plus small additions of salt, sodium nitrite, dye and Vitamin B1, together with sufficient water to give a fillable consistency.

* Composition variable, but generally as above.

**Bemax**

| Constituent | | |
|---|---|---|
| Vitamin B1 | 0.45 | Mg. per oz. |
| Vitamin B2 | 0.2 | |
| Nicotinic acid | 1.7 | |
| Vitamin B6 | 0.45 | |
| Vitamin E | 8.0 | |
| Manganese | 4.0 | |
| Iron | 2.7 | |
| Copper | 0.45 | |
| Protein | 27 | % |
| Carbohydrate | 50 | % |
| Fibre | 3(not more) | % |
| Calorific value | 104 | |

| Essential Amino Acids | Fresh wt.basis | 16% N.Basis |
|---|---|---|
| Arginine | 2.5 | 8.3 |
| Histidine | 0.9 | 3.0 |
| Lysine | 1.8 | 6.0 |
| Tryptophan | 0.3 | 1.0 |
| Phenlyalanine | 0.9 | 3.0 |
| Cystine | 0.3 | 1.0 |
| Methionine | 0.5 | 1.6 |
| Threonine | 1.2 | 4.0 |
| Leucine | 2.1 | 7.0 |
| iso-Leucine | 1.3 | 4.3 |
| Valine | 1.6 | 5.3 |

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# SOME EFFECTS OF HIGH TEMPERATURES ON THE PERCH PERCA FLUVIATILIS (LINNAEUS) AND THEIR IMPORTANCE IN INFLUENCING ITS DISTRIBUTION.

By A. H. Weatherley

SUMMARY.

The upper lethal temperature of the common perch Perca fluviatilis (L.) was determined experimentally in relation to acclimatization temperature. The information thus obtained was combined with knowledge of the distribution of the species in nature, to determine the extent to which high temperature limits its proximity to the equator. This analysis was aided by knowledge of the distribution of the very closely related yellow perch Perca flavescens (Mitchill) of North America. It was concluded that the distribution of both species is controlled by two principal factors. The first of these is their inability to live in streams, or sections thereof, with steep gradients. The second, high water temperature in midsummer, appears to exercise its effects in at least two extensive regions of the world. However, it appears almost certain that perch can live in waters in which midsummer temperatures may be only a degree or so below their upper lethal temperature. The conservative behaviour of perch, which inhibits free dispersal of these species, probably plays an important secondary role in

influencing the distribution pattern.

It is believed, then, that the more or less direct effects of temperature can control perch distribution, sometimes over a wide front, and that the mechanisms by which heat brings about its disabling or lethal effects are therefore of considerable importance for these two species. The major part of this thesis describes an investigation of some aspects of these mechanisms.

When specimens of P. fluviatilis were examined histologically it was found that exposure to (even moderately) high temperatures led to lymphocyte depletions in the head kidney and to cellular atrophy of the interrenal tissue. Higher levels of thermal exposure led, in addition, to more profound changes, so that when perch were exposed to temperatures of 28-30°C. for 1-2 weeks or more, even though elevation to such temperatures had been gradual, histological changes were found which were considered degenerative or incipiently so. The liver, renal tubules, muscles and possibly thyroid were among the affected tissues. Collectively these changes rather resemble the complex of adaptive and degenerative effects found in mammals exposed to prolonged noxious stimuli of many different kinds. Such changes in mammals, as well as many others of a non-histological character, were first described and named the General Adaptation Syndrome by the Canadian clinical investigator

H. Selye. During the last ten years or so a few workers had already suggested that the histological reactions of fish exposed to certain stresses were similar to those of mammals. There thus seemed good grounds for proposing that the response of perch to thermal stress might be integrated into this view, and that further experiments to test the validity of such a standpoint were indicated.

In the mammals the anterior pituitary governs the secretion of cortisone by the adrenal cortex in combatting stress. The teleost homologue of the adrenal cortex is the interrenal tissue, and the pronounced lability of the latter in the face of thermal stress led to the view that it was similarly involved. It was therefore reasoned that stressors other than heat (e.g. cold, anoxia, osmotic load) should alter the appearance of the perch interrenal in a similar fashion and bring about other changes resembling those produced by heat. Suitable experiments showed this to be the case, while the histological effects of injection of mammalian corticotrophin or "ACTH" (the secretion of the pituitary which activates the adrenal cortex) gave a further illustration of the involvement of the interrenal tissue. Furthermore, injections of corticotrophin were effective in raising the upper lethal temperature of perch by a statistically significant amount on several occasions. Injections

of cortisone, adrenalin and brei made from fish pituitaries and head kidneys were also given for reasons explained in detail in the thesis, though these were generally ineffective in offsetting thermal stress.

It was concluded that the interrenal tissue of perch is involved in combatting various forms of stress, of which heat stress is just a particular case. Degenerative changes accompanying exposure to more extreme stress are seen as indications that the adaptive potential of the fish (controlled at least in part by the interrenal) is about to be exceeded. It is of course not held that the interrenal tissue is the only line of defense against noxious stimuli; such a generalization is much beyond the scope of the present study.

Since it has been of such importance in this work a description is given of the vascular system and general morphology of the head kidney and kidney of the perch.